REPORT

NCI First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation: Report from the Committee on Disease-Specific Methods and Strategies for Monitoring Relapse following Allogeneic Stem Cell Transplantation. Part I: Methods, Acute Leukemias, and Myelodysplastic Syndromes

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Relapse has become the major cause of treatment failure after allogeneic stem cell transplantation. Outcome of patients with clinical relapse after transplantation generally remains poor, but intervention prior to florid relapse improves outcome for certain hematologic malignancies. To detect early relapse or minimal residual disease, sensitive methods such as molecular genetics, tumor-specific molecular primers, fluorescein in situ hybridization, and multiparameter flow cytometry (MFC) are commonly used after allogeneic stem cell transplantation to monitor patients, but not all of them are included in the commonly employed disease-specific response criteria. The highest sensitivity and specificity can be achieved by molecular monitoring of tumor- or patient-specific markers measured by polymerase chain reaction-based techniques, but not all diseases have such targets for monitoring. Similar high sensitivity can be achieved by determination of donor chimerism, but its specificity regarding detection of relapse is low and differs substantially among diseases. Here, we summarize the current knowledge about the utilization of such sensitive monitoring techniques based on tumor-specific markers and donor cell chimerism and how these methods might augment the standard definitions of posttransplant remission, persistence, progression, relapse, and the prediction of relapse. Critically important is the need for standardization of the different residual disease techniques and to assess the clinical relevance of minimal residual disease and chimerism surveillance in individual diseases, which in turn, must be followed by studies to assess the potential impact of specific interventional strategies.

Biol Blood Marrow Transplant 16: 1187-1211 (2010)

KEY WORDS: Allogeneic stem cell transplantation, Minimal residual disease, Chimerism, Acute leukemia, Myelodysplastic syndrome

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Financial disclosure: See Acknowledgments on page 1204.

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Received February 10, 2010; accepted June 6, 2010

1083-8791/$36.00
doi:10.1016/j.bbmt.2010.06.008

INTRODUCTION

Methodologic and technologic advances allow sensitive detection of minimal residual disease (MRD) and early recognition of recurrence of hematologic malignancies after allogeneic hematopoietic stem cell transplantation (alloHSCT). Importantly, intervention prior to florid relapse improves outcome for certain hematologic malignancies [1,2]. This manuscript by the *Workshop Committee on Disease-Specific Methods and Strategies for Monitoring Relapse following Allogeneic Stem Cell Transplantation* is divided into 2 parts and reviews disease-specific detection methods and available data with the use of such after alloHSCT. Given the critical importance to the goals of this *Workshop*, standard disease-specific response and relapse criteria are summarized. Outside of the alloHSCT setting, international working groups have developed standard diagnostic criteria that are widely employed in the definition of relapse for the different hematologic malignancies [3]. These are based on morphologic investigations of peripheral blood (PB) and/or bone marrow (BM), imaging, and/or specific laboratory findings. Given their critical importance to the goals of this *Workshop*, these criteria are summarized here. However, after alloHSCT, more sensitive methods such as molecular genetics, tumor-specific molecular primers, fluorescein in situ hybridization (FISH), multiparameter flow cytometry (MFC), and/or chimerism are commonly used to monitor patients with respect to relapse. Although some of these have clearly been shown to be predictive of outcome in specific diseases (eg, chronic myelogenous leukemia [CML]), and have become part of the standard criteria, assays for monitoring of disease status after alloHSCT have not yet been incorporated into the relapse definitions across all hematologic malignancies. Recommendations for the utilization of sensitive monitoring techniques to augment the standard definitions of posttransplant remission, persistence, progression, relapse, and the prediction of relapse are proposed, whenever possible, based on current evidence. It is anticipated that sensitive MRD detection will allow for earlier therapeutic intervention, and it is hoped that treatment prior to overt relapse may improve outcome of alloHSCT for hematologic malignancies. Critically important is the need to assess the clinical relevance of MRD surveillance in individual diseases, which in turn, must be followed by studies to assess the potential impact of specific interventional strategies. From the point of view of this *Workshop*, the use of these proposed definitions and methods should facilitate future studies of the natural history of relapse (*Committee on Epidemiology and Natural History of Relapse*), therapeutic interventions to prevent clinical relapse (*Committee on Strategies/Therapies Used to Prevent Relapse*), and the treatment of relapse (*Committee on Disease-Specific Treatment of Relapse*). Finally, major deficits and important questions for further clinical research will be addressed. In this first part, we focus on methods to detect and monitor disease response, persistence, progression, and relapse in acute leukemias and myelodysplastic syndrome (MDS), whereas in the forthcoming second part disease-specific monitoring for chronic leukemias, myeloproliferative neoplasms, and lymphoid malignancies will be reviewed.

METHODS TO DETECT AND MONITOR DISEASE RESPONSE, PERSISTENCE, PROGRESSION, AND RELAPSE

A wide variety of techniques are available to monitor residual disease after therapy, including in the posttransplant setting (Table 1), although the applicability varies by the specific disease subtype and the predictive value of each method is currently not well defined for most diseases. Some of these techniques are difficult to standardize, which is essential to the conduct of multicenter studies to assess the utility in the prediction and possible prevention of overt relapse.

Broadly, posttransplant monitoring of disease status is assured by 2 different methodologies: specific MRD detection and characterization of hematopoietic chimerism. The latter characterizes the origin of posttransplant hematopoiesis, whereas MRD detection measures the malignant clone directly. For each approach, a variety of techniques is available, although in general there have been more studies looking directly at markers of residual malignancy than of chimerism. Issues of applicability, standardization, sensitivity, and specificity are discussed separately for each technique.

Chromosome Banding Analysis

Classical chromosome banding analysis still plays an essential role in the biologic characterization and for prognostic predictions in many hematologic malignancies. Whenever possible, cultivation of metaphases is performed from BM instead of peripheral blood because of the higher proliferation rates and higher proportions of malignant cells in the setting of leukemia. In vitro proliferation of cells is supported by specific cytokines. Application of colchicine leads to cell cycle arrest shortly before metaphase preparation. Staining of chromosomes is performed with Giemsa-(G-), Quinacrin (Q-), or reverse (R-) banding techniques. Analysis of metaphases is improved by digital picture capture systems. The International System of Cytogenetic Nomenclature (ISCN) is used for the presentation of karyotypes. Analysis of 20 to 25 metaphases is required for valid results. With chromosome banding analysis, the whole karyotype can be illustrated within a single investigation. However,

Table 1. Diagnostic Methods to Monitor Residual Disease and Relapse of Hematologic Malignancies after alloHSCT

	Tumor Marker					Chimerism	
Method	Chromosome Banding	FISH	Flow Cytometry	Antigen Receptor PCR	Translocation or Other RT-PCR	XY FISH	qPCR/STR-PCR
Applicability	Subset of all types	Subset of all types	ALL; most AML; CLL; myeloma	ALL; lymphoma; CLL	CML; subset of ALL; subset of AML; subset of lymphoma	Sex mismatched alloHSCT	All types with differences in donor/recipient polymorphisms
Sensitivity	10^{-1}	10^{-2}	10^{-3}-10^{-4}	10^{-4}-10^{-5}	10^{-3}-10^{-6}	10^{-2}	10^{-3}-10^{-6}

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; PCR, polymerase chain reaction; qPCR, quantitative real-time PCR; STR, short tandem repeats.

chromosomal banding techniques are hampered by insufficient quality of BM samples (eg, myelofibrosis, recent history of total body irradiation [TBI]) as vital cells are required for metaphase culture. Moreover, chromosome banding analysis is limited to the detection of microscopically visible abnormalities. Sensitivity is further limited, as a maximum of 25 metaphases is usually evaluated within a single analysis. Further, full clarification of the chromosomal abnormalities often requires the combination with molecular cytogenetic methods using the FISH technique.

FISH

Diverse FISH techniques are available for the detection of submicroscopic alterations or for clarification of complex chromosomal changes including interphase FISH (IP-FISH), whole chromosome painting (WCP-FISH), 24-color-FISH (M-FISH or SKY), and comparative genomic hybridization (CGH). These techniques are all based on the hybridization of fluorescence-tagged probes to a specific chromosomal region. WCP-FISH and 24-color FISH can only be performed on metaphases. In contrast, FISH with loci specific probes or with probes for the centromeric regions can be performed on metaphases or interphase nuclei and thus do not require dividing cells. As interphase-FISH provides a higher sensitivity (100-200 cells can easily be evaluated) [4], the technique can be used for MRD [5], albeit not at a sensitivity approached with other techniques. Moreover, analysis is limited to the probes that have been chosen for the individual case [6].

MFC

The principle of detection of MRD by MFC rests on the property that leukemic cells have phenotypic properties that, although broadly similar to those of normal cells, often show subtle differences in antigen expression. Considerable effort has been put into characterizing the patterns of antigen expression in normal and regenerating hematopoietic differentiation. Comparison of specimens of leukemia to these normal templates has shown that most cases have "leukemia-associated immunophenotypes" (LAIPs). Typically, antigens normally expressed at a particular stage of maturation are over- or underexpressed in leukemic cells. Thus, with design of appropriate combinations of antigens in MFC analysis, leukemic cells can be recognized because they occupy areas of "empty space" on correlated plots of antigen expression [7-10]. By analyzing large numbers of cells (500,000 or more) with modern high-speed flow cytometers, it is possible to identify clusters of as few as 10 to 20 leukemic cells in a background of normal cells, allowing for a theoretic sensitivity approaching 10^{-5} cells. In practice, however, sensitivities are often limited to about 10^{-4} and may not even reach that in some cases.

Sensitivity of detection of residual leukemic cells varies between subtypes and depends upon the magnitude of the differences seen between leukemic cells and normal hematopoietic cells. Morever, the proportion of patients with informative phenotypes varies among different diseases. Studying larger panels of markers, either in higher order (more colors) flow cytometry or by adding additional tubes, will typically increase the ability to detect MRD. In B-lineage acute lymphoblastic leukemia (ALL), >90% of patients can be monitored with a relatively simple panel of antibodies [11,12], and similarly in B-chronic lymphocytic leukemia (CLL) a simple antibody combination is informative for detecting MRD at high sensitivity in nearly all cases [13]. By contrast, MRD detection in acute myelogenous leukemia (AML) is more complicated because leukemic signatures are often unique to an individual case, and custom panels are often designed to monitor particular patients [14-17]. Complicating detection of MRD by flow cytometry in acute leukemias is the fact that there may be phenotypic shifts following therapy, so looking only for those cells with the leukemia specific phenotype may underestimate, or even fail to identify residual leukemic cells [18-20]. Therefore, application of comprehensive antibody panels has been suggested for follow-up monitoring in AML patients. MRD in ALL can appear to undergo maturation following steroid therapy, and unless one is aware of this, MRD can be missed [21]. Phenotypic changes in AML are even more common, and the complexity of leukemic maturational patterns seen makes it difficult to clearly quantify leukemic populations even when some leukemic cells are identified. CLL and myeloma are clonal B

cell diseases that show light chain restriction, but in the MRD setting, identifying changes in the kappa/lambda ratio, or using other markers to identify subsets of cells that express only 1 light chain is generally not as sensitive as methods that employ the same kinds of aberrant antigen markers that are useful in MRD monitoring of acute leukemias [13,22].

Flow cytometric detection of MRD has a number of advantages over other methods. It is often relatively inexpensive compared to highly sensitive molecular methods, and results are obtained rapidly, often within hours or at most a day or 2, allowing for the design of studies that have an early therapeutic intervention based on MRD results. The method is particularly suited not only to detect, but also to quantify disease burden. This is of major importance to evaluate disease kinetics. A major disadvantage to MRD studies by flow in all diseases except CLL and multiple myeloma (MM) is that no international consensus has been reached on standardized panel design and data interpretation. Different panels of antibodies are used by different investigators and in different studies, and highly specialized experience is necessary for accurate interpretation of MRD results. There is even variablility in specimen processing, with some laboratories using ficolled separated cells [12] and others using cell lysis techniques [11,23]; some but not all of the latter techniques express MRD results as a percentage of mononuclear cells [11] to better match results obtained with ficolled samples. In contrast to molecular methods, there are not well-established protocols for demonstrating interlaboratory reproducibility. In B-lineage ALL, which is perhaps the best studied area in this regard, reproducibility is relatively high early in therapy when MRD burden is high and when there are the smallest number of contaminating normal B cell precursors. However, obtaining reproducibility at sensitivities of 10^{-5} or even 10^{-4} is very difficult [24]. Nevertheless, the clinical importance of MRD at such a low level has been well established in a variety of studies performed at 1 or few reference labs [11,23,25,26], suggesting that it is possible to measure clinically relevant MRD with that sensitivity.

Molecular Methods of Residual Disease Detection

The polymerase chain reaction (PCR) technique has proved to be an extremely robust method of high sensitivity and specificity with a wide range of applicability to residual disease detection depending upon the target chosen. PCR can be performed at the genomic level to look for DNA-based alterations, or alternatively reverse transcription PCR (RT-PCR) can be used to detect either structural or quantitative abnormalities in mRNA. Sensitivity and specificity of PCR can be improved by using "nested" PCR, in which a first reaction using primers directed at consensus sequences is followed by a second reaction using internal primers to further amplify DNA that was amplified in the first round. However, this "nested" PCR approach often yields unreliable quantitative results. Finally, PCR can either be performed using a qualitative or endpoint assay, or, now more commonly, quantitative real-time PCR (qPCR) can be used to quantify targets over a range of many orders of magnitude.

Minimal residual disease detection by antigen receptor PCR in lymphoid malignancies

Approaches for monitoring MRD in B- or T-lymphoid malignant diseases comprise techniques based on PCR to quantify tumor burden by disease-specific T cell receptor (TCR) or immunoglobulin (Ig) gene rearrangements [27]. Unique fingerprint-like sequences, assumed to be specific for each lymphoid cell, can be detected in the junctional regions of rearranged Ig and TCR genes. Cells of a lymphoid malignancy have a common clonal origin, with each cell having an identically rearranged Ig or TCR gene. Consequently, these gene rearrangements, located in the junctional regions of Ig and TCR genes, serve as specific targets for each leukemia and can be used for analysis of MRD [28,29]. At diagnosis, patient- and leukemia-specific clonal rearrangements are amplified by PCR. After this, the various disease specific rearrangements can be identified and selected [30]. After selection, PCR products are used for sequencing of the junctional regions. This specific sequence forms the basis for the design of junctional region specific oligonucleotides (allele-specific oligonucleotides [ASO]), required for leukemia, lymphoma, and myeloma-specific sensitive qPCR-based MRD analysis.

There are a number of potential pitfalls in the use of Ig and TCR clonality in MRD monitoring. Ig and TCR gene rearrangements in hematologic malignancies might be susceptible to subclone formation. Furthermore, secondary gene rearrangements could occur between diagnosis and relapse, which might cause false-negative MRD results. To reduce the number of false-negative MRD analyses in ALL, at least 2 Ig/TCR targets are used in clinical MRD studies [28,31]. Kreyenberg et al. [32] demonstrated that Ig and TCR gene rearrangements are stable markers for MRD in ALL after alloHSCT. Importantly, isolated false positive results in Ig-based qPCR systems have been observed at the time of intense B cell regeneration [33].

To assure comparability of MRD results between different laboratories, quality control and standardization are essential. A standardized approach for MRD analysis by qPCR has been implemented by the European Study Group on MRD detection in ALL (ESG-MRD-ALL), consisting of 30 laboratories worldwide. Furthermore, guidelines for the interpretation of qPCR-based MRD data have been developed.

Application of these guidelines allows identical interpretation of MRD data between different laboratories of the same MRD-based clinical protocol. Method and guidelines for its interpretation are precise to avoid both false-negative and false-positive results [31].

Fusion gene-specific targets and mRNA expression

Quantitative PCR can be used to monitor specific fusion gene transcripts as a sensitive indicator of MRD. Experience is most extensive in the setting of *BCR-ABL1* transcript monitoring for individuals with CML. The methodology used for identifying *BCR-ABL1* transcripts has evolved over the years. Initially to BCR-ABL transcripts were detected by single-step amplification or a 2-step "nested" amplification. In 1993, Cross et al. [34] developed a competitive semi-quantitative assay that expressed *BCR-ABL1* transcripts as a ratio (%) compared with normal *ABL* (*BCR-ABL1/ABL1* × 100). This method was adapted for qPCR when this technology became available [35,36]. Subsequently Hughes et al. [37], analyzing patients in the IRIS study, introduced the concept of logarithmic (log) reduction using as a baseline the laboratory-specific median *BCR-ABL1* transcript level taken from 30 patients.

For valid PCR data, it is imperative to optimize each stage of the procedure, including sample collection, RNA extraction, and quantitative PCR. The quality of the RNA is extremely important for reproducible data, and consistency in sample collection, tissue type, transportation, and storage conditions will maximize the accuracy and reliability of analysis. Standard protocols for *BCR-ABL1* PCR employ peripheral blood for MRD monitoring instead of BM aspirates.

It is highly desirable that a standardized international scale for measuring transcript levels be established, and it is probably preferable to move away from log reduction. An international collaborative group recently published recommendations whereby the standardized baseline would be determined using a reference material, and the absolute *BCR-ABL1* value representing a major molecular response would be standardized at 0.1%. A value of 1.0% would be approximately equivalent to cytogenetic complete remission (CR) [38,39].

MRD monitoring by PCR techniques has also been utilized in the assessment of treatment response and for the early detection of relapse in other leukemias with sensitivities of 10^{-4} to 10^{-6} [40-48]. Specific reciprocal gene rearrangements (eg, *PML/RARa*, *RUNX1-RUNX1T1* = *AML1-ETO*, *CBFB-MYH11*), intragenic duplications (eg, *MLL-PTD*, *FLT3-ITD*), and other mutation types (eg, alterations of the *NPM1* gene) can be used for monitoring MRD in AML [49]. However, reciprocal gene fusions are applicable in a minority of AML patients only. *NPM1* mutations represent ideal targets for MRD monitoring by qPCR in adults [50,51], but have a low prevalence in pediatric AML (8%-10%). Thus, MRD monitoring based on molecular markers has so far been realized only for subgroups of patients with AML. In 50% of patients with myelofibrosis and in 5%-10% of patients with MDS, the *JAK2*V617F mutation can be monitored by sensitive qPCR [52]. Furthermore, the *MPL*W515L/K mutation can be detected and monitored in about 5% of myelofibrosis patients [53].

The transcription factor *WT1*, originally described as a tumor suppressor gene, is a key molecule for neoplastic proliferation in a large number of hematologic malignancies, making it suitable both as an universal MRD maker and as a potential target for therapeutic strategies (eg, vaccination) [45,54-58]. *WT1* has been shown to be expressed in the majority of adult and pediatric cases of acute leukemias, CML-blast phase, MDS, and both T- and B-lineage non-Hodgkin lymphoma (NHL), although the 3-4 $\log_{10}$ overexpression necessary to make it a reasonable MRD marker is only seen in a subset of cases [59-65]. The frequency and degree of WT1 expression has been shown to correlate with MDS disease progression [66]. WT1 expression, quantified by qPCR, has been evaluated as a marker for risk stratification and for MRD detection in AML [44-48,66-69]. However, there are contradictory reports about the utility of WT1 overexpression to monitor MRD during frontline chemotherapy and after alloHSCT [45,46,56,57,70,71].

Chimerism

Analysis of chimerism allows monitoring of hematopoietic cells from donor and recipient origin after alloHSCT to determine engraftment as well as detection of imminent graft rejection and may also serve as an indicator for recurrence of the underlying malignancy. Because chimerism analyses were first performed, many different methods have been developed and implemented, all following the same basic principle using differences in polymorphic genetic markers and their products. These methods include cytogenetics [72,73], red cell, or HLA phenotyping [74-76], restriction fragment-length polymorphism (RFLP) analysis [77-80], and FISH of sex chromosomes [81-86]. A major limitation of these different techniques is that they are time consuming and not applicable to all patients.

The breakthrough for clinical applicability came when the PCR technique was developed [87] and also utilized for investigation of chimerism [88-94]. During the 1990s, these analyses were mainly performed by the amplification of variable number of tandem repeats (VNTR) or by the characterization of short tandem repeat (STR) markers. Fluorescent labeling of the primers and resolution of PCR products by capillary electrophoresis allowed accurate

quantification of the degree of mixed chimerism. Semiautomated PCR analyses using the appropriate hardware permitted a high sample throughput [93,95-98] facilitiating the study of chimerism in all patients in short time intervals starting early after transplantation. Thus, accurate monitoring of engraftment as well as surveillance of impending graft rejection and imminent relapse has become possible.

Furthermore, a qPCR assay for the analysis of the SRY gene on the Y chromosome has been established, which allows the identification of male DNA in the background of female DNA at very low levels [99]. This method is able to detect 1 male cell in the background of 100,000 female cells [100,101] increasing sensitivity enormously.

Over the past years, qPCR-based chimerism techniques aiming at the amplification of sequence polymorphisms (SPs) were established. SPs represent the most frequent genetic variation, occur on an average of 1.3 kilobases in the human genome, are mostly biallelic, and differ predominantly only in 1 single nucleotide, denoted by single nucleotide polymorphisms (SNPs) [102-104]. Alizadeh et al. [105] were the first to report a set of 11 biallelic polymorphisms using qPCR amplification for chimerism analysis. The limit of detection for the minor cell population (0.1%) was higher than in STR-PCR. This study was followed by others demonstrating the possibility of accurate characterization of chimerism by qPCR based on polymorphisms [106,107]. In contrast to STR-PCR, where virtually all recipient/donor pairs could be characterized, only 90% of donor/recipients could be discriminated by this assay. Furthermore, this qPCR technique showed less quantitative accuracy with a variation coefficient of 30%-50% for higher autologous signals [105,106] compared to a variation coefficient of 5% when applying STR-PCR systems [95,97,108-111]. However, this did not hamper analysis when only low levels of mixed chimerism were needed to be quantified. Large prospective trials utilizing this qPCR method are needed to determine whether the clinical impact of chimerism analysis can be improved. For the time being, fluorescence-based PCR amplification of STR seems to be the gold standard method for posttransplant chimerism surveillance and the great majority of the major studies published to date have used this technique. Despite the increasing sensitivity of methods to determine chimerism, the clinical utility of this approach is limited. In most hematologic malignancies, chimerism is not a tumor-specific marker, and finding recipient cells does not necessarily indicate disease recurrence. The persistence or reappearance of recipient cells, detected by molecular methods, could reflect the survival of leukemic blasts, survival of normal host hematopoiesis, or a combination of both phenomena. Surviving host-derived hematopoietic cells could promote reemergence of the malignant clone by inhibiting immunocompetent donor- derived effectors.

Hematologic relapse was preceded by reappearing host-derived hematopoiesis in the mononuclear cell fraction in CML patients [112]. In consequence, the graft-versus-leukemia (GVL) effect could be weakend by a state of mixed chimerism [113]. In several early reports, it remained unclear if a state of mixed chimerism in patients with an acute leukemia was associated with an increased risk of relapse. The dynamic process of the evolution of chimerism was described in the 1990s, and it was concluded that monitoring of chimerism should be performed serially and in short intervals of time. The specificity in this regard is higher in diseases that originate from a stem- or progenitor cell (eg, CML), in contrast to malignancies that originate from a later cell stage of development (eg, CLL or myeloma), in which case the usefulness of chimerism to detect MRD or relapse is low. This lack of specificity might be overcome by performing chimerism on specific subsets of cells in some diseases (see later) [114,115]. Here, chimerism is performed on cell subtypes after isolation by cell sorting or enrichment (eg, CD138 for myeloma, CD34 for AML and MDS). Finding chimerism in a population enriched for tumor cells may increase the likelihood that it is a true marker of residual disease. Chimerism analysis may also have value, as it provides information about the alloreactivity and/or tolerance induction of the graft and thus may serve as a prognostic factor independent of its role as a marker for MRD.

Imaging

Imaging studies are not generally useful as MRD markers in leukemias or myeloproliferative neoplasms, but they play a role in lymphomas and MM. In MM, lytic lesions are generally diagnosed by radiographic analysis. One weakness of radiographic detection is that it may reveal lytic disease only when over 30% of the trabecular bone is lost [116]. Because of these limitations, computed tomography (CT) or magnetic resonance imaging (MRI) have been used to increase the sensitivity and specificity. CT is currently the most commonly used means for restaging patients with lymphoma [117,118]. However, CT lacks functional information, which impedes identification of disease in normal-sized organs. 18-F-fluoro-2-deoxyglucose (FDG) positron emission tomography (PET) may be an alternative to CT [119]. MRI is also utilized for bone and soft tissue reevaluation in specific situations.

CT

The mainstay of imaging studies for lymphoma has always been CT scans performed at various intervals. With current CT scanners, lymph nodes of 5 mm or

less in diameter can be detected. Extranodal involvement is typically demonstrated on a CT by organomegaly, abnormal masses, structural changes, or abnormal contrast enhancement. One problem with CT scans is that they have limited specificity, as many different processes can produce enlarged lymph nodes, even in patients with lymphoma [120], and a biopsy may be necessary to confirm residual disease. For use with MM patients, CT imaging is superior to that of standard radiography and reveals more lesions compared with conventional radiology [121].

MRI

MRI has high spatial resolution and excellent soft-tissue contrast that makes it an ideal tool for the detection of parenchymal and osseous lesions. However, because of long imaging time, limited availability, and extensive costs, MRI was previously used only as a tool to image limited anatomic areas of the body. Recent improvements in MRI technology have resulted in the availability of sufficiently fast and diagnostic sequences for whole-body (WB) MRI. However, there is no standard WB-MRI protocol for staging malignant lymphoma at this time. MRI provides superior soft-tissue contrast to CT. As with CT, assessment of the nodal involvement is based on size criteria, and biopsy is needed for final confirmation of relapse. The MRI sequences that are most informative for detection of bone lesions that may be observed in myeloma and lymphomas, are the T1-weighted, the T1-weighted with fat suppression and gadolinium contrast, the T2-weighted with fat suppression, and the short-time inversion recovery (STIR) images. The STIR images are particularly sensitive in detecting myelomatous lesions [122].

PET

PET is based on the use of positron-emitting radiopharmaceuticals and the detection in coincidence of the 2 nearly collinear 511-keV photons emitted following positron annihilation with an electron. The increased glycolytic rate of malignant cells is the rationale behind the common use of FDG as a radiotracer [123]. More recently FDG-PET and CT scans have been integrated, which provides both functional and anatomic information [124]. FDG-PET positivity is not restricted to malignancy. For example, uptake can be seen with inflammation and infection resulting in "false-positive" results in the setting of cancer evaluations. FDG-PET scanning has been integrated into standard staging criteria for lymphomas [125]. FDG-PET/CT can detect Richter's transformation of CLL to diffuse large B cell lymphoma (DLBCL) with a high sensitivity and a high negative predictive value [126]. Standardized FDG-PET and PET/CT imaging procedure guidelines have been published to improve image quality, reporting, and common imaging standards, and may enable the use of semiquantitative techniques such as the SUV (standardized uptake value) in assessing response to therapy among centers worldwide [127]. PERCIST 1.0 is a proposed FDG-PET/CT response imaging criteria in solid tumors. This proposes imaging methology and image analysis to assess response semiquantiatively or quantitatively. It is intended as a starting point for use FDG-PET in clinical trials and structured quantitative reporting, which may be particularly useful when assessing the activity of newer therapies that stabilize disease [128]. The standardization of imaging and response criteria could be a good step in establishing the use or lack of utility of FDG-PET/CT in early/interim (after 1-3 cycles of therapy) response monitoring or early PET response adapted therapy trials [129]. Multiple novel alternative clinical PET tracers have been developed, but the greatest experience outside of FDG-PET has been with 3′-deoxy-3′-[18F] fluorothymidine (FLT), which is an analog for thymidine used to image tumor proliferation. Early pilot studies using FLT-PET/CT appear to be very promising in early response evaluation in lymphoma [130].

DISEASE-SPECIFIC DEFINITIONS AND MONITORING OF RELAPSE AFTER alloHSCT

Standard diagnostic criteria have been established to define response and relapse for the hematologic malignancies. These criteria have historically been based on morphologic BM investigations (eg, blast count in acute leukemias), imaging methods (eg, occurrence of new lymph nodes on FDG-PET scans for NHL), and/or specific laboratory findings (eg, increased paraprotein by immunofixation and electrophoresis in MM). Recently, more sensitive methods have been utilized to assess patients for disease response. Some, but not all of these approaches, have been integrated into response criteria definitions for various hematologic malignancies. Herein, we propose criteria for incorporation of currently available methodologies in the definitions for disease response, persistence, progression, relapse, and the prediction of relapse after alloHSCT.

AML

According to the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia, remission criteria in AML are not solely based on morphology, but also include cyto- and molecular genetic as well as flow cytometric data. Cytomorphology and hematologic parameters retain a central position (Table 2) [131]. However, very few follow-up

studies have been performed to specifically assess these criteria in the posttransplant period.

These criteria could also be integrated into the assessment of remission in the posttransplant period. Because of the limited sensitivity of chromosomal banding [132], interphase FISH seems to be a more suitable parameter for the assessment of the cytogenetic remission status. There are only few studies that assess the prognostic value of interphase FISH after AML therapy [5], and even fewer studies addressing this issue in the posttransplant period. In pediatric cases with MDS or AML Fuehrer et al. [6] showed that clonal cytogenetic markers, as assessed by interphase FISH (eg, monosomy 7), reappeared at relapse after alloHSCT and could be followed after second transplant. Metaphase FISH proved useful for MRD diagnostics following standard chemotherapy or alloHSCT in an analysis of 22 AML patients performed by El-Rifai et al. [133], as all but 1 patient with persisting or increasing levels of abnormal cells relapsed.

Chimerism

Both in AML and MDS, several studies demonstrated the relevance of chimerism, and especially its kinetics, for the prediction of relapse. Using a semiquantive STR method in 81 pediatric alloHSCT recipients with AML, Bader et al. [134] found a relapse rate of 47% in those with increasing mixed chimerism (ie, increase of recipient cells) in contrast to 13% in patients with complete or decreasing mixed chimerism ($P < .005$) [134]. In the study of Huisman et al. [135], chimerism status was monitored by STR-PCR in T and non-T cell subsets in 96 patients with AML after myeloablative (MA) or reduced-intensity conditioning (RIC). Stable complete donor chimerism was detected in 56% of patients of the MA group in contrast to 12% in the RIC group. In samples taken between 1-6 months posttransplant, complete donor chimerism or decreasing mixed chimerism (ie, decrease of recipient cells) was significantly associated with a lower relapse risk (31% versus 83%) and mortality (38% versus 83%, $P < .001$) when compared to increasing mixed chimerism. Similar results were shown by Zeiser et al. [136], who performed STR-PCR both with conventional and CD34-specific chimerism methods in 168 patients with AML or MDS after MAC. With $CD34^+$-specific chimerism, decrease of donor alleles was detected at a median of 30 days before the clinical manifestation of relapse. The relapse rate was 89% in patients with mixed chimerism in contrast to 6% in those with complete donor chimerism.

Molecular genetics

Molecular mutations. A variety of genetic markers are available for MRD studies and have been investigated within a standard chemotherapy setting for their potential to guide therapeutic decisions. In this setting, MRD measurement is well established for the reciprocal rearrangements t(15;17)/*PML-RARA*, inv(16)/*CBFB-MYH11*, and t(8;21)/*RUNX1-RUNX1T1*. These are found in approximately 20% of de novo AML cases and confer a favorable prognosis for patients treated with standard chemotherapy. Ratios of aberrant gene expression after consolidation therapy and at diagnosis were demonstrated to correlate significantly with prognosis [137], and distinct thresholds of transcript copy numbers were determined to be associated with an increased relapse risk [42,41]. After standard chemotherapy, the interval from the increase of fusion transcripts to morphological manifestation of relapse was 3-6 months. With respect to the posttransplant period, Elmaagacli et al. [138] followed 8 patients with inv(16)/*CBFB-MYH11* with reverse transcription (RT-) PCR. The *CBFB-MYH11* transcript was not detectable in 6 of 7 patients who remained in stable remission post-alloHSCT, whereas relapse occurred in 1 of 2 patients who had been positive 3 months after alloHSCT.

In patients with de novo AML, the prognostically favorable nucleophosmin (*NPM1*) mutations are detectable in approximately 35% of overall AML cases. In normal karyotype AML, frequencies about 55% were reported. qPCR or high melting resolution PCR ("HRM") can be used to detect the most frequent *NPM1* mutation subtypes A, B, and D (Figure 1). Some studies demonstrated a correlation of the mutation load before and after chemotherapy with outcomes [139-141]. In most studies in the standard therapeutic setting, *NPM1* mutations provided high stability during follow-up [50,139,140], whereas 1 study described loss of the mutation in 2 of 21 relapsing patients [142]. In an analysis involving 13 stem cell recipients with a history of *NPM1* mutated AML, the achievement of PCR negativity after alloHSCT was a precondition for stable remission

Table 2. Response Criteria in AML According to an International Working Group [131]

Criteria	Complete Remission	Relapse
Morphologic and hematologic	BM blasts <5%; thrombocytes $\geq 100 \times 10^9$/L; neutrophils $\geq 1.0 \times 10^9$/L	Reappearance of blasts after CR (BM: ≥5%; PB)
Cytogenetic	Disappearance of previous cytogenetic alteration	Reappearance of cytogenetic alteration
Molecular	Disappearance of molecular mutation	Reappearance of molecular mutation
Flow cytometric	Disappearance of cells with previously determined LAIP	Reappearance of cells with LAIP

AML indicates acute myeloid leukemia; BM, bone marrow; CR, complete remission; PB, peripheral blood; LAIP, leukemia associated immunophenotype.

[49], whereas relapse after alloHSCT was preceded by an increase of the *NPM1* mutation level with mean intervals of 24 days (range: 12 - 38 days) in all but 1 case. The short interval between MRD detection and relapse in *NPM1* mutated AML implies that very frequent monitoring (eg, weekly intervals) would be necessary if this marker were to be used for early therapeutic intervention to prevent hematologic relapse. The more favorable prognosis of patients with isolated *NPM1* mutations and a normal karyotype suggests that lower frequencies of this mutation might be expected in the alloHSCT setting. Posttransplant MRD studies for this marker would likely be more important in patients with coincident *NPM1* mutations and the prognostically unfavorable internal tandem duplication of the *FLT3* gene (*FLT3*-ITD) [143,144].

FLT3-ITDs are seen in 20%-25% of all AML cases and in approximately 40% of cases with normal karyotypes [145]. *FLT3*-ITD have variable lengths and different starting points; thus, qPCR monitoring requires the design of patient-specific primers [146]. Some studies have indicated that the *FLT3*-ITD mutation was unstable, with loss of the mutation in nearly a fifth of relapsed patients, limiting the validity of this marker for MRD monitoring [147]. However, others have reported instability rates of <5% [148]. Integration of *FLT3*-ITD in posttransplantation monitoring has been suggested [149], but is still controversial because of the potential for instability of the mutation [150]. Consequently, a combination with a second MRD strategy (eg, *NPM1* mutations [49,151]), or with MFC, might be recommended for *FLT3* mutated AML.

Mutations of the tyrosine kinase domain of the *FLT3* gene (*FLT3*-TKD) are less frequent than the *FLT3*-ITD in AML. They occur in 6%-8% of all AML cases with normal karyotypes [152-156]. The impact on prognosis remains incompletely defined [157,156]. Scholl et al. [146] demonstrated that quantitative assessment during follow-up with qPCR is feasible as the *FLT3*-TKD mutations are represented by single nucleotide base exchanges. Quantitative follow-up of the *FLT3*-ITD and the *FLT3*-TKD was performed with qPCR in 4 stem cell recipients with a history of *FLT3* mutated AML and showed a significant association of relapse to posttransplant PCR positivity, whereas disease-free survival (DFS) was associated with attainment of PCR negativity [157].

Partial tandem duplications of the *MLL* gene (*MLL*-PTD) represent another promising marker for

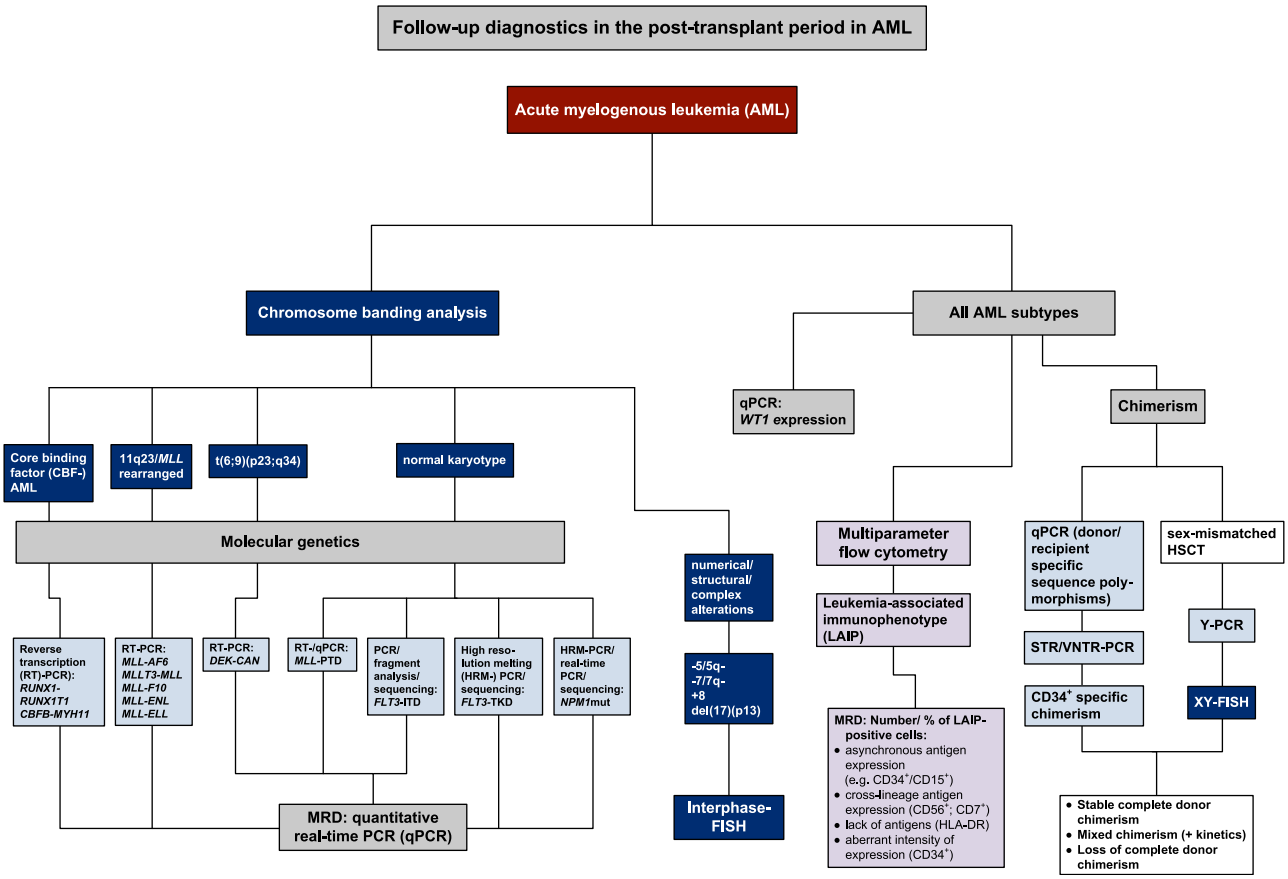

Figure 1. Proposed algorithm for follow-up diagnostics in the posttransplant period in AML.

posttransplant monitoring in AML. These prognostically unfavorable gain-of-function mutations [158] occur in 5%-10% of patients with a normal karyotype [159-162]. MRD studies with qPCR demonstrated that the amount of reduction of mutated cells was prognostically relevant in the standard therapeutic setting. Molecular relapse preceded clinical manifestations by several weeks [163]. To date, no study has been performed with this marker specifically in the posttransplant period.

Single gene expression. Monitoring of the expression of the *WT1* gene represents another option for posttransplant MRD detection in patients with AML [46,150]. Using qPCR in patients with AML, ALL, or advanced phases of CML, Ogawa et al. [164] were able to determine thresholds above which relapse was predictable within 40 days after alloHSCT. There was a 100% relapse probability at an expression level of $1\text{-}5 \times 10^{-2}$, whereas the relapse risk was <1% with expression levels $<4 \times 10^{-4}$. Relapsed patients who responded to adoptive immunotherapy by donor lymphocyte infusion (DLI) or withdrawal of immunosuppression showed a significantly longer doubling time of *WT1* expression compared to patients who were refractory to these approaches. Doubling time of *WT1* transcripts <13 days was predictive for failure of treatment. Candoni et al. [165] described full concordance between *WT1* expression levels and the remission status of AML before and after alloHSCT. Jacobsohn et al. [166] evaluated the prognostic significance of pretransplant WT1 gene expression as a marker of MRD in 36 pediatric patients with AML. Elevated WT1 gene expression before alloHSCT was predictive of posttransplant relapse and lower 5-year event-free survival (EFS). After alloHSCT, 76% of patients with high pretransplant WT1 expression relapsed, in contrast none of those with low WT1 expression.

Multicenter studies have been initiated to develop standardization of *WT1* measurement (eg, by qRT-PCR normalized to the *ABL* gene) [58]. However, the physiologic background expression of *WT1* in healthy individuals has to be considered. Barragan et al. [141] compared *WT1* expression and monitoring of the *NPM1* mutation load in 24 AML patients and found a strong correlation between both markers ($P < .001$), but with different disappearance kinetics. *WT1* expression showed rapid decrease after induction therapy, but residual levels were maintained during CR, whereas *NPM1* mutations showed a mild reduction after induction, but they were undetectable in long-term survivors. Thus, assessment by qPCR of the *NPM1* mutation load was superior to the assay for *WT1* quantification because of the specificity for leukemic cells and higher levels of expression at presentation.

Expression of other genes such as *BAALC* (brain and acute leukemia, cytoplasmic) [167] might be investigated for posttransplant monitoring in normal karyotype AML as well, although to date there are no data on their usefulness for this specific purpose. Thus, a variety of additional genetic markers should be investigated for their potential utility after alloHSCT, particularly those with an adverse impact on prognosis as they are likely to be overrepresented in the transplant setting. Efforts should continue to develop algorithms for monitoring patients with AML in the posttransplant period (Figure 1).

MFC

The value of immunophenotyping by MFC for the determination of the remission status in AML has been confirmed in many studies in the setting of standard therapy [131]. The decrease of cells with the specific LAIP from diagnosis to the end of induction therapy showed significant correlation with the remission rates and long-term prognosis [168-170], and the reduction of aberrant cells to a threshold of <0.1% after chemotherapy was associated with improved survival rates [171]. Only very few studies [172] have addressed the value of MFC specifically in the posttransplantation period, but it was shown that MFC was able to discriminate leukemic cells from regeneration blasts after alloHSCT [173]. In an individual patient with AML, withdrawal of immunosuppression was guided by the results of MFC, as there was an increase of cells with the LAIP 2 months posttransplant. This was followed by a subsequent reduction of aberrant cells [174]. Perez-Simon et al. [175] performed MFC in 13 patients with AML or MDS who had received RIC-alloHSCT. Persistence of cells with the LAIP within 21-56 days posttransplant was associated with a relapse risk close to 60%, whereas all patients with a negative MRD status in this period achieved stable remission. Diez-Campelo et al. [176] performed MRD monitoring by MFC in 41 alloHSCT recipients with AML or MDS. A cutoff level of 10^{-3} malignant cells at 100 days posttransplant was able to discriminate different risk populations. The 4-year overall survival was 73% in patients with a low MRD level versus 25% among patients with high MRD level at this time point, and EFS after 4 years was 74% versus 17% ($P = .01$) for the same 2 groups. The authors thus proposed that MRD monitoring with MFC has relevance for the therapeutic decisions in the early posttransplant period.

It should be emphasized that 1 advantage of MFC is the option to perform this technique in AML patients without evidence of a specific genetic marker. However, the frequency of changes of the LAIP in patients relapsing after alloHSCT is unknown, whereas relapse after standard therapy was associated with loss of the previous LAIP in approximately 25% of cases [177]. Thus, further evaluation is needed to evaluate the status of MFC for AML patients in the posttransplant period.

MDS

As in AML, standard criteria for the assessment of remission in MDS have been developed and revised by an international committee [178]. These new criteria integrate clinical, morphologic, cytogenetic, and hematologic parameters (Table 3). Few studies have evaluated MRD diagnostics in patients with MDS in the posttransplant period.

Molecular Markers

WT1 expression was shown to increase during leukemic transformation of MDS and to decrease following chemotherapy or alloHSCT [66]. In a study from Cilloni et al. [57] that included 131 patients with MDS, 65% of BM and 78% of PB samples from patients with refractory anemia (RA) showed *WT1* overexpression, whereas 100% of samples from patients with either refractory anemia with excess blasts (RAEB) or secondary AML (sAML) samples *WT1* transcript amounts greater than the level observed in healthy volunteers. Thus, there was significant correlation between the *WT1* expression level and the IPSS score. Similar results were reported by Patmasiriwat et al. [179], who described increased *WT1* levels in advanced MDS cases, but not in RA. Bader et al. [69] found significantly higher mean *WT1* expression levels in pediatric patients with RAEB and juvenile myelomonocytic leukemia (JMML) when compared to expression levels of healthy donors ($P < .001$). However, single samples showed overlap between patients and healthy individuals.

Tamura et al. [180] reported on a patient of 14 years of age who had a diagnosis of RAEB with an increase of *WT1* expression to 16,000 copies/μg RNA in peripheral blood after alloHSCT. Withdrawal of immunosuppression was followed by transient and self-limiting skin graft-versus-host disease (GVHD) and gradual decrease of *WT1* expression during the next months. Sixteen months after alloHSCT, the patient was in CR, whereas *WT1* expression was below the detection level. However, because it is difficult to differentiate clearly between phyisological and aberrant gene expression, the usefulness of *WT1* expression for posttransplant monitoring of myeloid malignancies remains controversial.

Table 3. Revised Criteria for Assessment of Remission in MDS According to an International Working Group [180]

Criteria	Response
Morphologic and hematologic	Complete remission (CR): bone marrow blasts <5% without dysplasia, hemoglobin ≥11 g/dL, platelets ≥100 × 10^9/L, neutrophils ≥1.5 × 10^9/L.
	Partial remission (PR): reduction of blasts by at least 50% or achievement of lower risk category than prior to treatment.
Cytogenetic	Major cytogenetic response: disappearance of a cytogenetic abnormality.
	Minor cytogenetic response: ≥50% reduction of abnormal metaphases.

MDS indicates myelodysplastic syndrome.

Only a few studies have investigated molecular mutations in MDS. Most frequent are intragenic mutations of the *RUNX1/AML1* gene [181,182]. *FLT3*-ITD occurs in 3%-5% of MDS patients [183,185]. Mutations of the *NRAS* protooncogene were identified in 6%-10% of all MDS cases. In contrast to AML, *NPM1* mutations are rarely if ever seen in MDS [186]. Thus, the proportion of MDS patients who can be characterized with molecular markers is lower than in AML. However, as *FLT3*-ITD or *NRAS* mutations were demonstrated to increase during leukemic transformation [184,185], the selection of high-risk MDS cases for alloHSCT might be associated with an overrepresentation of these markers when compared to standard therapy cohorts. So far, no study has specifically determined the frequency of these molecular markers in the allo-transplant setting.

MFC

Several studies have demonstrated that MFC has potential diagnostic utility in MDS. Frequently, the side-scatter (SSC) signal is reduced because of lower granularity of granulocytes. Aberrant expression patterns of CD13 and CD16 on granulocytes or lack of CD71 on erythrocytes can also be characteristic [187,188]. The progression of MDS to higher stages was shown to be accompanied by an increase in the flow cytometric scores [189], which correlated with the International Prognostic Scoring System and prognosis [190]. At this time, no flow cytometric study has been performed specifically in the posttransplant period in MDS patients. Whether the frequent finding of bone marrow dysplasia in the posttransplant period will be an obstacle for this approach, remains to be clarified.

In conclusion, considering that molecular MRDs markers are available or under development for specific AML subgroups only, and the lack of MRD markers in most MDS patients, surveillance of chimerism represents the most broadly applicable molecular tool in patients with these disorders. Monitoring of the kinetics of chimerism in short intervals is clearly superior to the interpretation of a single measurement. In addition, the potential of $CD34^+$ specific chimerism for myeloid disorders seems worthy of further evaluation.

ALL

Standard definitions for response and relapse are based on clinicopathologic evaluation of peripheral blood, BM, cerebrospinal fluid, and potential extramedullary sites of involvement. Response criteria rely most commonly on blood, marrow, and cerebral spinal fluid (CSF) cell counts and morphology (Table 4). From the standpoint of long-term outcome, CR has

Table 4a. Response Criteria in ALL

Complete remission (CR): a CR requires that *all* of the following be recorded concurrently:
- <5% marrow leukemia blast cells (M1).
- No circulating blasts.
- Absolute neutrophil count (neutrophils and bands) $\geq 1.0 \times 10^9$/L.
- Platelet count $\geq 100 \times 10^9$/L.
- Adequate bone marrow cellularity with trilineage hematopoiesis.
- Absence of extramedullary manifestations of disease (eg, CNS1).
- No evidence of recurrence of ALL for at least 4 weeks.

*Morphologic CR with incomplete blood count recovery (CRi): above CR criteria without specified blood counts.
Cytogenetic CR (CRc): in addition to above CR criteria, reversion to normal karyotype for those with previously detected cytogenetic abnormality.
Molecular CR (CRm): in addition to above CRc criteria, normalization of previously detected molecular cytogenetic abnormality.
*Partial response (PR): requires that *all* of the following be recorded concurrently:
- Decrease in the percentage of marrow blasts, absolute peripheral blast count, and extramedullary disease by at least 50%.
- ≤25% marrow leukemia blast cells.
- Absolute neutrophil count (neutrophils and bands) $\geq 1.0 \times 10^9$/L.
- Platelet count $\geq 100 \times 10^9$/L.

*Stable disease (SD)
- Criteria not met for CR, PR, or progression.

Progressive disease
- An increase of at least 25% in the absolute number of circulating or bone marrow leukemic blasts or extramedullary disease burden; *or*
- Development of new extramedullary disease.

Relapsed disease
- The reappearance of leukemia blast cells in the blood or the bone marrow (≥25%) or in any other extramedullary site after a CR with confirmation of lymphoid blasts by morphology and flow cytometry, PCR for antigen receptor loci or fusion genes, or cytogenetics/FISH; *or*
- Increase to >25% blasts in the marrow after a PR.
- Importantly, isolated extramedullary relapses (eg, CNS) are considered relapse from a diagnostic standpoint, although these are commonly approached differently in terms of therapy.

CNS indicates central nervous system; ALL, acute lymphoblastic leukemia.
*Additional definitions sometimes employed in the context of clinical trial.

historically been considered the only meaningful goal and endpoint. However, criteria for CR with incomplete blood count recovery (CRi), partial response, and stable disease are sometimes utilized in the context of early phase clinical trials (Table 4).

Importantly, the standard definition for relapse in patients with CR1 (>25% BM blasts) is insensitive for use after allogeneic haemaotopoietic stem cell transplantation, as detailed later.

Prognostic Significance of MRD in Nontransplant Studies

Multiple studies support the independent prognostic value of MRD measurements in pediatric and adult patients with B- and T-lineage ALL [11,191-219]. It has been well demonstrated that MRD positivity (MFC and PCR-based molecular methods) after induction and consolidation [201,202,204] correlates with the risk of relapse. Most studies have employed BM and, in general, this is the preferred sample source; however, in T-ALL, in contrast to B-ALL, levels of MRD in blood and marrow correlate closely so that PB may be utilized in MRD detection [208,209]. However, measurement in PB may also provide useful prognostic information in B-ALL [208], especially early in therapy during periods of BM aplasia (ie, day 8) [11]. Furthermore, the risk of relapse appears to be proportional to the level of MRD, which in some studies was found to be the most powerful prognostic factor for relapse in multivariate analyses [26,191-195,203,206,210]. The kinetics of the decrease in MRD level has been shown to be more predictive than MRD analyzed at 1 time point [26,194]. Consequently, MRD detection has been utilized in the stratification for several ALL treatment protocols [196,197,206] and to evaluate the effectiveness of alloHSCT or alternative treatment approaches [194].

Prognostic Significance of MRD at the Time of Transplant

Pediatric studies

Numerous studies have shown on a retrospective basis that the MRD status prior to conditioning was the strongest predictor for posttransplant relapse [211,212,213]. A British pediatric study in Bristol reported that an MRD load of greater than 10^{-3} pretransplant was associated with poor survival. In contrast, a portion of patients with low level disease burden ($<10^{-3}$) revealed a more favorable outcome, and MRD-negative children had the best chance of survival [210].

A retrospective study in Germany including 45 children reconfirmed these results, applying the same semiquantitative PCR approach. Those patients, entering transplantation with high-level MRD ($>10^{-3}$) were rarely cured. Interestingly, residual disease could be eradicated by posttransplant immunotherapy in some of these high-risk patients [206].

Recently Bader et al. [214] published prospective data on MRD analysis in 91 children with relapsed

Table 4b. Bone Marrow Classification

	Blast Percentage (at least 200 nucleated cells counted)
M1	<5%
M2	5%-25%
M3	>25%

Bone Marrow Classification in ALL.

ALL in second or higher remission prior to alloHSCT within the ALL-REZ BFM Study Group. The probability of EFS and cumulative incidence of relapse (CIR) in patients with MRD $\geq 10^{-4}$ were 0.27 and 0.57, respectively, compared to 0.60 and 0.13 in 46 patients with MRD $<10^{-4}$ (EFS: $P = .004$; CIR: $P < .001$). Multivariate Cox regression analysis revealed MRD as the only independent parameter predictive for EFS. These findings proved the clinical significance of MRD analysis prior to alloHSCT as a predictor for posttransplant outcome in a large prospective study. Based on this, new strategies with modified alloHSCT procedures will be evaluated within the ALL-BFM trials.

Adult studies

There are few studies examining the prognostic significance of MRD detection prior to and following alloHSCT for adults with standard risk B-cell ALL. In several older studies [198-201], semiquantitative PCR analysis failed to detect a strong association of MRD in CR1 prior to alloHSCT with posttransplant DFS. In 1 of these studies [201], there was no significant difference in pretransplant MRD detection for the 14 patients who were in continuous CR after transplant; 8 were MRD positive and 6 were MRD negative prior to alloHSCT. However, there was an association with pretransplant MRD and risk of relapse for a cohort of patients who underwent autologous HSCT in CR. Twenty-one of 23 patients (91%) who were MRD-negative prior to autologous HSCT remained in long-term remission following transplant. In contrast, 6 of 7 patients (86%) who tested MRD-positive prior to autologous HSCT relapsed following the transplant ($P = .005$). In this same study, posttransplant MRD detection was strongly associated with relapse-free survival (RFS) for the 19 patients undergoing alloHSCT. There were no relapses in patients who remained MRD-negative following alloHSCT, whereas both patients with detectable MRD following transplant relapsed.

Table 4c. Central Nervous System (CNS) Classification

	Cerebrospinal Fluid Cell Count and Cytology
CNS1	Absence of blasts on cytospin preparation.
CNS2	<5 white blood cells/μL and cytospin positive for blasts.
CNS3	≥5 white blood cells/μL and cytospin positive for blasts.

Bone Marrow Classification in ALL.

A more recent study using qPCR examined the clinical impact of MRD detection in 43 adults with high-risk ALL who underwent alloHSCT [215]. The group was heterogeneous in regard to disease subtype and status, conditioning, and donor type. Overall survival (OS) at 36 months was 48%. For patients who were PCR-negative prior to transplant, 80% were alive at 36 months in contrast to 49% survival for PCR-positive patients ($P = .17$). For the same patients, the cumulative incidence of relapse was 0% for PCR-negative and 46% for PCR-positive patients ($P = .03$). The relapse rate calculated according to the molecular results at 100 days following alloHSCT confirmed that the achievement or maintenance of a PCR-negative status within 3 months following transplantation was significantly associated with a lower incidence of relapse at 36 months posttransplant (7% versus 80%, $P = .0006$). In a multivariate analysis to investigate the role of clinical findings including disease status prior to transplant, cytogenetics, immunophenotype, age, and pretransplant MRD level, only molecular CR before conditioning proved to be a significant predictor of the achievement of MRD negativity at 100 days after transplantation.

Bassan et al. [206] recently demonstrated the utility of risk-adapted therapy based on MRD measurements for adults with ALL. MRD measurements taken at weeks 10, 16, and 22 of therapy using qPCR were used to stratify treatment for adults receiving intensive postremission chemotherapy for ALL in CR1. Of 280 patients registered to the trial, 112 were evaluable for MRD at the end of consolidation therapy. Of these, 58 had no measurable MRD and 54 were MRD-positive. Five-year OS was 75%, and DFS was 72% for the MRD-negative group in comparison with 33% OS and 14% DFS ($P = .001$) for the MRD-positive group (regardless of pretreatment risk assignment based on clinical, immunophenotypic, and cytogenetic features). They further stratified postremission treatment based on MRD. Those patients who were MRD-positive at weeks 16 through 22 were assigned to receive alloHSCT in CR1 if an available donor was identified, or in the absence of a donor, to an intensification of postremission chemotherapy. Those who were MRD-negative went on to receive traditional maintenance therapy. Thirty-six of 54 (67%) MRD-positive cases proceeded to alloHSCT or to the intensified chemotherapy phase. There was a clear improvement in 4-year DFS for these 36 patients estimated at 33% in comparison to no survivors among the 18 MRD-positive patients who did not receive either intensified treatment approach. Notably, MRD status after the intensified treatment further defined prognosis for this group. For the patients who achieved a MRD-negative state following alloHSCT or intensified chemotherapy, the DFS was 51%; for those who were remained MRD-positive, there were

Table 5. Published Studies on the Use of Chimerism and MRD in alloHSCT for ALL

Chimerism

Late 1980s to mid 1990s

Author	Patients (n)	Graft (n)	T Cell Depleted	Interval of Investigations	Methods	State of Chimerism		Relapses	
						MC	CC	MC	CC
Schattenberg et al., Blood 1989 [74]	29	29	yes	6, 12 months	Red cell phenotype Cytogenetics RFLP	19/29		3/19	
Van Leeuwen et al. Blood 1993 [245]	53	46 7	yes no	2, 6, 12 months	VNTR	23/53	30/53	8/23	10/30
Roy et al. Blood 1990 [246]	43	43	yes	0.5, 1, 3, 6, 9, 12, 18, 24 months	RFLP	22/43	21/43	11/22	6/21
Lawler et al. Blood 1991 [89]	32	12 20	yes no	not given	VNTR and STR	10/12 8/20	2/12 12/20	4/10 2/8	0/2 0/12
Bader et al. BMT 1998 [247]	55	55	no	Weekly	VNTR and STR	18/36 1 autologous recovery		Increasing MC: relapse	

Chimerism with Intervention

2003 to 2008

Author	Patients (n)	Diagnosis	Interval of Investigations	Methods	Relapses
Formánková et al. Haematologica 2003 [229]	54	ALL, AML, CML, and MDS Children	weekly to +100; monthly	STR	MC associated with rejection and relapse, immunotherapy was possible
Gorczyñska et al. BMT 2004 [230]	14	ALL, AML Children	weekly to +100; monthly	STR	Increasing MC could be converted by immunotherapy to CC
Bader et al. JCO 2004 [235]	163	ALL Children	weekly to +100; monthly	STR	MC associated with rejection and relapse, immunotherapy was possible
Horn et al. BMT 2008 [248]	20	ALL, AML Children	1, 3, 6, 12 months; in MC bi-weekly	STR	MC associated with relapse, immunotherapy was not possible

Pretransplant MRD

Retrospective Studies

Author	Patients (n)	Diagnosis	Interval of Investigations	Methods	Survival According to MRD Status
Knechtli et al. Blood 1998 [213]	64	ALL	prior to conditioning	Ig/TCR PCR	high level positive—0% low level positive—36% negative—73%
Bader et al. Leukemia 2002 [211]	41	ALL	prior to conditioning	Ig/TCR PCR	high level positive—23% low level poitive—48% negative—78%
Uzunel et al. Blood 2001 [249]	30	ALL	prior to conditioning	Ig/TCR PCR	high level positive—47% low level positive—50% negative—100%
Sramkova et al. Ped Blood Cancer 2007 [250]	25	ALL	prior to conditioning	Ig/TCR PCR	positive—0% negative—94%
Prospective Studies					
Bader et al. JCO 2009 [214]	91	ALL	prior to conditioning	Ig/TCR PCR	MRD $\geq 10^{-4}$: EFS 27%; CIR 57% MRD $<10^{-4}$: EFS 60%; CIR 13%
Posttransplant MRD					
Retrospective Studies					
Knechtli et al. BJH 1998 [237]	68	ALL	up to 24 months posttransplant	Ig/TCR PCR	relapse—88% positive remission—22% positive
Uzunel et al. BJH 2003 [239]	23	ALL	24 months	Ig/TCR PCR	MRD positive associated with relapse
Sanchez et al. BJH 2002 [238]	40	ALL	day 30, 60, 90, every 2 to 3 months	Flow cytometry	positive—33% negative—74%

(Continued)

Table 5. (*Continued*)

Pretransplant MRD					
Prospective Studies					
Author	Patients (n)	Diagnosis	Interval of Investigations	Methods	Survival According to MRD Status
Bader et al. BMT 2009 [240]	92	ALL	day 30, 60, 100, 200, 365 posttransplant	Ig/TCR PCR	MRD $\geq 10^{-4}$: EFS 9%; RFS 11% MRD $<10^{-4}$: EFS 48%; RFS 62%
Mortuza et al. JCO 2002 [201]	19	ALL (B-lineage)	1 to 20 months	Ig/TCR PCR (semiquant.)	positive – 0% negative – 100%
Spinelli et al. Haematologica 2007 [215]	37	ALL	day 100	Ig/TCR or fusion gene PCR (quantitative)	positive $>10^{-4}$—20% negative—93%
Bassan et al. Blood 2009 [206]	18	ALL (All patients were PCR positive prior to transplant)	not defined	Ig/TCR PCR	positive $>10^{-4}$ —0% negative—50%

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CIR, cumulative incidence of relapse; CC, complete chimerism; CML, chronic myelogenous leukemia; EFS, event-free survival; Ig, immunoglobulin; MC, mixed chimerism; MDS, myelodysplastic syndromes; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RFS, relapse-free survival; STR, short tandem repeats; TCR, T cell receptor; VNTR, variable number tandem repeats.

no long-term survivors at 4 years. These data suggest that allocation to standard maintenance therapy for patients with MRD-negative status following consolidation results in excellent DFS and that intensification therapy with alloHSCT or repeated cycles of intensification chemotherapy can salvage some very high-risk patients (MRD-positive at the end of consolidation) who would otherwise relapse with near certainty.

Pretransplant MRD monitoring in Philadelphia chromosome-positive ALL

MRD detection prior to transplant for Philadelphia chromosome positive (Ph+) ALL is a good predictor of RFS after transplant [216-220]. The addition of targeted tyrosine kinase inhibition with imatinib to frontline therapy of Ph+ ALL has resulted in the ability to significantly reduce or eradicate MRD during the first months of treatment using sensitive qRT-PCR assays [221-224]. A number of recent studies have demonstrated that achievement of a molecular CR as assessed by qPCR immediately prior to the time of transplant correlates with improved DFS and OS after alloHSCT. In 1 of the larger studies, when imatinib was added to frontline therapy, 82% of the patients became PCR negative and the majority was able to proceed to transplant [225]. With a median follow-up of 25 months following transplant, outcome of these patients appeared to be improved with a DFS of 78% in comparison to studies in the preimatinib era where DFS following alloHSCT for adults with Ph+ ALL was in the 30% to 50% range. The kinetics of MRD eradication prior to alloHSCT may also be an important prognostic feature. Lee et al. [226] studied MRD in 41 patients with Ph+ ALL who were treated with combination chemotherapy induction followed by 4 weeks of imatinib therapy. They found that 36 of 41 patients achieved at least a 3-log reduction of MRD following the first 4 weeks of treatment with imatinib. This early reduction in MRD was identified as the most powerful predictor of lower relapse rate following alloHSCT. For patients with at least a 3-log reduction in MRD, the relapse rate following alloHSCT was 12% in comparison to the other patients who had a relapse rate of 45% ($P = .011$) [227]. These data, similar to the pediatric data presented before, suggest that pretransplant MRD detection in adults with ALL may be an important guide to early posttransplant treatment to attempt to avert clinical relapse.

Role of MRD Monitoring and Chimerism Assessment following alloHSCT for ALL

Pediatric and adult studies (Table 5)

Assessment of chimerism using the semiquantitative STR-PCR approach, which analyzed microsatellite regions, has demonstrated that ALL patients with rapid increase of mixed chimerism were at the highest risk of relapse post-alloHSCT [113,228-231]. The analysis of cell subpopulations in acute leukemia patients revealed a potential difference between children and adults. Guimond et al. [232] demonstrated that mixed chimerism in T and natural killer (NK) cells was frequently detected in children with relapsed leukemia but not in pediatric patients, who remained in remission. Furthermore this phenomenon was not observed in relapsed adult patients.

The clinical relevance of a state of increasing mixed chimerism as a risk factor for relapse in patients with acute leukemia was demonstrated by Barrios et al. in

2003 [233]. However, a group in Germany showed a more complicated relationship between chimerism and relapse [234]. They showed that the persistence of mixed chimerism in the early-phase posttransplant as predominantly caused by normal recipient-derived hematopoiesis. The reappearance of leukemic blasts was preceded by an increase of this recipient-derived normal hematopoiesis, denoted by increasing recipient chimerism. These results promoted the hypothesis that mixed chimerism weakened the clinical GVL effect, which was thought to be mediated by donor-derived alloreactive effectors in acute leukemia patients. These results formed the basis for several clinical trials to study the efficacy of relapse prevention by preemptive immunotherapeutic intervention based on the analysis of chimerism in acute leukemia patients [229,230].

By applying STR-based analysis of chimerism at defined regular intervals posttransplant, in 2004, Bader et al. [235] defined a subset of children with impending relapse, applying STR-based analysis of chimerism at defined regular intervals posttransplant. Beyond that, prevention of overt relapse was possible in a portion of patients by preemptive immunotherapeutic intervention (withdrawal of immunosuppression or administration of DLI), although impending relapse was not diagnosed in all patients because of the short time interval between the conversion of chimerism and the occurrence of relapse in some patients.

Importantly, analysis of chimerism by conventional STR-PCR does not incorporate the potential to detect MRD because of its limited sensitivity (approximately 1%). Thus, the decision to start immunotherapy should not be based exclusively on the state of chimerism, because this might result in a delay of onset of beneficial interventional therapy.

A German retrospective analysis including patients who had received DLI based on a state of increasing mixed chimerism also measured the MRD burden, applying qPCR (Ig/TCR rearrangements). It was demonstrated that those patients with an MRD burden of $>10^{-3}$ did not benefit from interventional therapy, whereas patients with an MRD load $<10^{-3}$ at initiation of immunotherapy showed a survival rate just below 40% at 2 years [236].

The significance of MRD analysis posttransplant has only been investigated in a few prospective studies. In 1998, Knechtli et al. [237] showed that persistent MRD positivity represented an unfavorable predictor for EFS. Here, MRD was assessed by a semiquantitative dot blot hypridization approach. These initial results could be confirmed later in a smaller cohort of patients by immunophenotyping of antigens associated with leukemia [238] and by molecular qPCR based approaches [175,215,239]. These trials all showed MRD to be a predictor for poor posttransplant outcome.

Recently, the ALL-REZ BFM Study Group monitored MRD in 92 pediatric ALL patients after alloHSCT to highlight patients with highest risk for relapse to whom preemptive treatment might be offered. The probability of EFS and RFS for MRD-negative patients was 0.55 and 0.77, respectively, compared to 0.48 and 0.62 in MRD-positive patients with $<10^{-4}$ and compared to 0.09 and 0.11 in MRD-positive patients with $\geq 10^{-4}$, respectively (EFS: $P < .005$; RFS: $P < .001$). Patients who remained persistently MRD-negative showed a RFS of 0.78 compared to 0.5 in patients who became MRD-negative, and compared to only 0.1 in patients who developed an increase of MRD $\geq 10^{-4}$ and of 0 in patients with MRD levels remaining permanently $>10^{-4}$ ($P < .001$). Consequently, patients with reappearing or persisting MRD load at a level of $\geq 10^{-4}$ faced the highest risk for subsequent relapse and may therefore be candidates for further interventional therapy [240].

MRD by molecular Ig/TCR rearrangements is impossible in patients lacking a disease-specific marker. In these cases, the status of chimerism can be used as a surrogate marker for MRD if it is analyzed in specific cell subpopulations. Thiede et al. [241] demonstrated that a state of mixed chimerism in the $CD34^+$ cell fraction predicted relapse in PB in ALL and AML patients. In 1998, Winiarski et al. [242] characterized chimerism in cell subsets, which carried the leukemic phenotype. A remarkable correlation between the presence of MRD and a state of mixed chimerism in the respective cell subpopulation was detected. However, large prospective trials proving the predictive value of mixed chimerism in cell subsets have not been published yet.

Taken together, posttransplant serial monitoring of chimerism by conventional STR-PCR in PB allowed for the identification of patients with impending relapse. Therefore, frequent chimerism analyses are indispensable within the first 200 posttransplant days, when most relapses occur.

The combination of characterization of chimerism and analysis of MRD ensures the documentation of engraftment and surveillance of posttransplant state of remission. These approaches provide a rational basis for individual immunotherapeutic intervention to prevent the recurrence of the underlying malignancy. However, it should be noted that the clinical importance of MRD in ALL patients after allogeneic stem cell transplantation could not be verified in some studies [210].

Posttransplant MRD monitoring in Ph+ ALL

Beginning in the late 1990s, studies to assess MRD status following alloHSCT have provided intriguing information about the risk of relapse in Ph+ ALL [216-219,243]. First, using qualitative RT-PCR testing with sensitivities reported in the 10^{-5} to 10^{-6} range, a number of studies found that patients who were consistently PCR negative following alloHSCT were unlikely to relapse. Conversely, patients in whom MRD was detected after transplant were at

very high risk of relapse. In the largest published series, Radich et al. [218,219] found that the relative risk (RR) of relapse was significantly higher for patients with detectable MRD following transplantation than for those without detectable *BCR-ABL1* transcripts (RR: 5.7; $P = .025$). The prognostic significance of the PCR assay remained after controlling for other clinical variables (eg, stem cell source, GVHD) that could influence relapse risk. Interestingly, the genetic context of MRD may also be of relevance. In this study, Radich et al. noted that the risk of relapse was greatest for MRD-positive patients with a p190 *BCR-ABL1* transcript in comparison to patients who had detectable p210 *BCR-ABL1* transcripts after transplant.

Use of imatinib after alloHSCT appears to be useful for eradication of MRD in some patients. Wassmann et al. [244] showed that initiation of imatinib for MRD-positive patients after alloHSCT was associated with a rapid molecular CR and prolonged DFS in 14 of 27 treated patients. There have also been anecdotal reports of initiation of targeted tyrosine kinase inhibitor therapy at the time of clinical relapse with reinstitution of donor chimersim and achievement of prolonged remission with or without DLI.

Summary and Future Directions: Risk-Adapted Therapy of ALL Based on MRD

Taken together, all the studies suggest that MRD measurements in patients with ALL taken in the first 4-20 weeks of postremission therapy have strong prognostic significance and can identify patients with both a good and poor prognosis. Similarly, detection of pretransplant MRD in pediatric and some adult studies is highly predictive of relapse following alloHSCT and, coupled with posttransplant MRD evaluation, may guide early posttransplant intervention such as early withdrawal of immunosuppression, administration of DLI, or addition of posttransplant maintenance therapy (eg, targeted tyrosine kinase inhibiation for Ph+ ALL).

Thus, there are ample data demonstrating that MRD monitoring is a powerful prognostic tool in pediatric and adult ALL and may be used to risk-adapt treatment. The introduction of novel treatment strategies to eradicate MRD early during treatment should be a goal of future trials and may eventually obviate the need for alloHSCT. Characterization of MRD before transplantation defines those patients at highest risk for relapse after alloHSCT. Importantly, although it appears that MRD detection can facilitate the decision to advise an allogeneic transplant in CR1, the outcome for MRD+ positive patients, even with the most aggressive approaches currently available, is still relatively poor. After alloHSCT, MRD measurements can be used to guide novel therapeutic interventions and assess their efficacy. MRD monitoring allows the detection of impending relapse in a substantial

Table 6. Response and Relapse Definitions after alloHSCT—Application of Monitoring Methodologies

Disease	Definition of Complete Remission	Definition of Relapse	Molecular Marker	Cytogenetics	Chimerism	Imaging	Flow Cytometry
AML/MDS	IWG	IWG	Molecular mutations	Chromosome banding analysis, FISH	PCR or VNTR/STR		4-8 color flow
Applicable	All patients	All patients	Subgroups	Subgroups	All patients	Not applicable	All patients
Comment	Well established.	Well established, but less sensitive.	Expansion of MRD marker panel for posttransplant monitoring in AML (eg, *NPM1* mutations) or MDS (eg, *RUNX1/AML1* mutations).*	No standardization for MRD monitoring, useful for specific aberrations.*	Well-established, lack of specificity: investigation of $CD34^+$ specific chimerism*; and standardization of techniques.		Few studies.*
ALL	Less than 5% blasts in BM	More than 25% blasts in BM	TCR- and Ig- Gene rearrangement	Chromosome banding analysis, FISH	PCR or VNTR/STR		4-6 color flow
Applicable	All patients	All patients	90% of all patients	Subgroups	All patients	Not applicable	> 95% of patients
Comment			ASO primer: 80%–90% of patients. Ig VD]: Most patients. *BCR-ABL1*: All Ph+ ALL.	Clinical not important for MRD assessment.	Gold standard: Singleplex PCR with fluorescent labelled STR primers. Importantly: product resolution using capillary electrophoresis.		Sensitivity in B-lineage ALL is limited after SCT because of large numbers of hematogones.

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; Flow, multiparameter flow cytometry; MDS, myelodysplastic syndromes; qPCR, quantitative real-time PCR; STR, short tandem repeats; VNTR, variable number tandem repeat.
*Further studies needed.

percentage of children undergoing transplantation for ALL. These analyses have recently served as the basis for treatment intervention to avoid graft rejection, maintain engraftment, and treat-imminent relapse.

SUMMARY, CONCLUSIONS, AND FUTURE RESEARCH

Because the intention of alloHSCT is to cure the underlying hematologic malignancy, and because there is increasing evidence that minimal disease after alloHSCT may be eradicated by immunotherapeutic approaches such as DLI, monitoring of disease is of great importance. The current definitions of remission and relapse utilized to evaluate most hematologic malignancies during upfront therapy lack sufficient sensitivity for use after alloHSCT. Flow cytometry, molecular methods, and new imaging modalities have been investigated in recent years and can substiantially increase the sensitivity of disease detection up to 10^{-4} to 10^{-6}. The highest sensitivity and specificity can be achieved by molecular monitoring of tumor- or patient-specific markers measured by PCR, but not all diseases have such targets for monitoring. Similar sensitivity can be achieved by determination of donor chimerism, but its specificity regarding detection of relapse is low and differs substantially among diseases. A higher specificity might be obtained by lineage-specific donor chimerism, but there are only a few such studies with limited number of patients.

Critically important is the need for standardization of the different residual disease techniques. Table 6 summarizes the different methods of MRD detection in the monitoring of ALL, AML, and MDS, and the relative pros and cons of use of these methods after alloHSCT. Further clinical trials to assess the utility of these techniques in each disease entity are also mandatory. The predictive value of posttransplant MRD and chimerism, and the optimal timepoints to assess kinetic changes in these measurements remains to be determined across all hematologic malignancies. Subsequent studies must then be performed to evaluate the efficacy of MRD- and chimerism-guided therapeutic interventions designed to prevent overt relapse. Thus, critical objectives for future studies in this area should include the following:

1. Standardize measurement of molecular markers for each hematologic malignancy for which alloHSCT is employed.
2. Determine the optimal frequency for monitoring MRD and chimerism after alloHSCT.
3. Define kinetic changes in MRD and chimerism that occur after alloHSCT and establish criteria for remission and relapse that incorporate measurement of these molecular markers in the definition of response and remission after alloHSCT.
4. Assess the efficacy of interventional strategies based changes in MRD and/or chimerism to prevent clinical relapse.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

REFERENCES

1. Raanani P, Dazzi F, Sohal J, et al. The rate and kinetics of molecular response to donor leucocyte transfusions in chronic myeloid leukaemia patients treated for relapse after allogeneic bone marrow transplantation. *Br J Haematol*. 1997;99:945-950.
2. Bader P, Klingebiel T, Schaudt A, et al. Prevention of relapse in pediatric patients with acute leukemias and MDS after allogeneic SCT by early immunotherapy initiated on the basis of increasing mixed chimerism: a single center experience of 12 children. *Leukemia*. 1999;13:2079-2086.
3. Baccarani M, Cortes J, Pane F, et al. European LeukemiaNet. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol*. 2009;27:6041-6051.
4. Haferlach T, Bacher U, Kern W, et al. Diagnostic pathways in acute leukemias: a proposal for a multimodal approach. *Ann Hematol*. 2007;86:311-327.
5. Bacher U, Kern W, Schoch C, et al. Evaluation of complete disease remission in acute myeloid leukemia: a prospective study based on cytomorphology, interphase fluorescence in situ hybridization, and immunophenotyping during follow-up in patients with acute myeloid leukemia. *Cancer*. 2006;106:839-847.
6. Fuehrer M, Gerusel-Bleck M, Konstantopoulos N, et al. FISH analysis of native smears from bone marrow and blood for the monitoring of chimerism and clonal markers after stem cell transplantation in children. *Int J Mol Med*. 2005; 15:291-297.
7. Dworzak MN, Fritsch G, Panzer-Grumayer ER, Mann G, Gadner H. Detection of residual disease in pediatric B-cell precursor acute lymphoblastic leukemia by comparative phenotype mapping: method and significance. *Leuk Lymphoma*. 2000;38:295-308.
8. Lucio P, Gaipa G, van Lochem EG, et al. BIOMED-I concerted action report: flow cytometric immunophenotyping of precursor B-ALL with standardized triple-stainings. BIOMED-1 Concerted Action Investigation of Minimal Residual Disease in Acute Leukemia: International Standardization and Clinical Evaluation. *Leukemia*. 2001;15:1185-1192.
9. Porwit-MacDonald A, Bjorklund E, Lucio P, et al. BIOMED-1 concerted action report: flow cytometric characterization of CD7+ cell subsets in normal bone marrow as a basis for the diagnosis and follow-up of T cell acute lymphoblastic leukemia (T-ALL). *Leukemia*. 2000;14:816-825.
10. Weir EG, Cowan K, LeBeau P, Borowitz MJ. A limited antibody panel can distinguish B-precursor acute lymphoblastic leukemia from normal B precursors with four color flow cytometry: implications for residual disease detection. *Leukemia*. 1999;13:558-567.
11. Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood*. 2008;111: 5477-5485.
12. Campana D, Coustan-Smith E. Minimal residual disease studies by flow cytometry in acute leukemia. *Acta Haematol*. 2004;112:8-15.

13. Rawstron AC, Villamor N, Ritgen M, et al. International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia. *Leukemia*. 2007;21:956-964.
14. Al-Mawali A, Gillis D, Lewis I. the role of multiparameter flow cytometry for detection of minimal residual disease in acute myeloid leukemia. *Am J Clin Pathol*. 2009;131:16-26.
15. Kern W, Danhauser-Riedl S, Ratei R, et al. Detection of minimal residual disease in unselected patients with acute myeloid leukemia using multiparameter flow cytometry for definition of leukemia-associated immunophenotypes and determination of their frequencies in normal bone marrow. *Haematologica*. 2003; 88:646-653.
16. Macedo A, Orfao A, Gonzalez M, et al. Immunological detection of blast cell subpopulations in acute myeloblastic leukemia at diagnosis: implications for minimal residual disease studies. *Leukemia*. 1995;9:993-998.
17. San Miguel JF, Martinez A, Macedo A, et al. Immunophenotyping investigation of minimal residual disease is a useful approach for predicting relapse in acute myeloid leukemia patients. *Blood*. 1997;90:2465-2470.
18. Borowitz MJ, Pullen DJ, Winick N, et al. Comparison of diagnostic and relapse flow cytometry phenotypes in childhood acute lymphoblastic leukemia: implications for residual disease detection: a report from the children's oncology group. *Cytometry B Clin Cytom*. 2005;68:18-24.
19. Tomova A, Babusikova O. Shifts in expression of immunological cell markers in relapsed acute leukemia. *Neoplasma*. 2001; 48:164-168.
20. Zeleznikova T, Babusikova O. The impact of cell heterogeneity and immunophenotypic changes on monitoring minimal residual disease in acute myeloid leukemia. *Neoplasma*. 2006; 53:500-506.
21. Gaipa G, Basso G, Maglia O, et al. Drug-induced immunophenotypic modulation in childhood ALL: implications for minimal residual disease detection. *Leukemia*. 2005;19:49-56.
22. Rawstron AC, Orfao A, Beksac M, et al. Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. *Haematologica*. 2008;93:431-438.
23. Dworzak MN, Froschl G, Printz D, et al. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. *Blood*. 2002;99:1952-1958.
24. Dworzak MN, Gaipa G, Ratei R, et al. Standardization of flow cytometric minimal residual disease evaluation in acute lymphoblastic leukemia: multicentric assessment is feasible. *Cytometry B Clin Cytom*. 2008;74:331-340.
25. Bjorklund E, Mazur J, Soderhall S, et al. Flow cytometric follow-up of minimal residual disease in bone marrow gives prognostic information in children with acute lymphoblastic leukemia. *Leukemia*. 2003;17:138-148.
26. Coustan-Smith E, Sancho J, Hancock ML, et al. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. *Blood*. 2000;96:2691-2696.
27. Gabert J, Beillard E, van der Velden V, et al. Standardization and quality control studies of "real-time" quantitative reverse transcription transcription polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia—a Europe Against Cancer program. *Leukemia*. 2003;17:2318-2357.
28. van der Velden VH, Hochhaus A, Cazzaniga G, et al. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. *Leukemia*. 2003;17:1013-1034.
29. van der Velden V, van Dongen JJ. MRD detection in acute lymphoblastic leukemia patients using Ig/TCR gene rearrangements as targets for real-time quantitative PCR. *Methods Mol Biol*. 2009;538:115-150.
30. Pongers-Willemse MJ, Seriu T, Stolz F, et al. Primers and protocols for standardized detection of minimal residual disease in acute lymphoblastic leukemia using immunoglobulin and T cell receptor gene rearrangements and TAL1 deletions as PCR targets: report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia*. 1999;13:110-118.
31. van der Velden VH, Cazzaniga G, Schrauder A, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia*. 2007;21:604-611.
32. Kreyenberg H, Eckert C, Yarkin Y, et al. Immunoglobulin and T-cell receptor gene rearrangements as PCR-based targets are stable markers for monitoring minimal residual disease in acute lymphoblastic leukemia after stem cell transplantation. *Leukemia*. 2009;23:1355-1358.
33. Fronkova E, Muzikova K, Mejstrikova E, et al. B-cell reconstitution after allogeneic SCT impairs minimal residual disease monitoring in children with ALL. *Bone Marrow Transplant*. 2008;42:187-196.
34. Cross NC, Hughes TP, Feng L, et al. Minimal residual disease after allogeneic bone marrow transplantation for chronic myeloid leukaemia in first chronic phase: correlations with acute graft-versus-host disease and relapse. *Br J Haematol*. 1993;84: 67-74.
35. Hochhaus A, Lin F, Reiter A, et al. Quantification of residual disease in chronic myelogenous leukemia patients on interferon-alpha therapy by competitive polymerase chain reaction. *Blood*. 1996;87:1549-1555.
36. Cross NC, Feng L, Chase A, et al. Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. *Blood*. 1993;82:1929-1936.
37. Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med*. 2003;349:1423-1432.
38. Muller MC, Saglio G, Lin F, et al. An international study to standardize the detection and quantitation of BCR-ABL transcripts from stabilized peripheral blood preparations by quantitative RT-PCR. *Haematologica*. 2007;92:970-973.
39. Cross NCP, Hughes TP, Hochhaus A, et al. International standardisation of quantitative real-time RT-PCR for BCR-ABL. *Leukemia Res*. 2008;32:505-506.
40. Gallagher RE, Yeap BY, Bi W, et al. Quantitative real-time RT-PCR analysis of PML-RAR alpha mRNA in acute promyelocytic leukemia: assessment of prognostic significance in adult patients from intergroup protocol 0129. *Blood*. 2003;101: 2521-2528.
41. Marcucci G, Caligiuri MA, Dohner H, et al. Quantification of CBFbeta/MYH11 fusion transcript by real time RT-PCR in patients with INV(16) acute myeloid leukemia. *Leukemia*. 2001;15:1072-1080.
42. Krauter J, Gorlich K, Ottmann O, et al. Prognostic value of minimal residual disease quantification by real-time reverse transcription transcription polymerase chain reaction in patients with core binding factor leukemias. *J Clin Oncol*. 2003; 21:4413-4422.
43. Schnittger S, Weisser M, Schoch C, et al. New score predicting for prognosis in PML-RARA+, AML1-ETO+, or CBFB MYH11+ acute myeloid leukemia based on quantification of fusion transcripts. *Blood*. 2003;102:2746-2755.
44. Cilloni D, Saglio G. WT1 as a universal marker for minimal residual disease detection and quantification in myeloid leukemias and in myelodysplastic syndrome. *Acta Haematol*. 2004; 112:79-84.
45. Lapillonne H, Renneville A, Auvrignon A, et al. High WT1 expression after induction therapy predicts high risk of relapse and death in pediatric acute myeloid leukemia. *J Clin Oncol*. 2006;24:1507-1515.
46. Weisser M, Kern W, Rauhut S, et al. Prognostic impact of RT-PCR-based quantification of WT1 gene expression during MRD monitoring of acute myeloid leukemia. *Leukemia*. 2005;19:1416-1423.

47. Barragan E, Cervera J, Bolufer P, et al. Prognostic implications of Wilms' tumor gene (WT1) expression in patients with de novo acute myeloid leukemia. *Haematologica*. 2004;89:926-933.
48. Steinbach D, Schramm A, Eggert A, et al. Identification of a set of seven genes for the monitoring of minimal residual disease in pediatric acute myeloid leukemia. *Clin Cancer Res*. 2006;12: 2434-2441.
49. Bacher U, Badbaran A, Fehse B, et al. Quantitative monitoring of NPM1 mutations provides a valid minimal residual disease parameter following allogeneic stem cell transplantation. *Exp Hematol*. 2009;37:135-142.
50. Schnittger S, Kern W, Tschulik C, et al. Minimal residual disease levels assessed by NPM1 mutation-specific RQ-PCR provide important prognostic information in AML. *Blood*. 2009; 114:2220-2231.
51. Hollink IH, Zwaan CM, Zimmermann M, et al. Favorable prognostic impact of NPM1 gene mutations in childhood acute myeloid leukemia, with emphasis on cytogenetically normal AML. *Leukemia*. 2009;23:262-270.
52. Kröger N, Badbaran A, Holler E, et al. Monitoring of the JAK2-V617F mutation by highly sensitive quantitative real-time PCR after allogeneic stem cell transplantation in patients with myelofibrosis. *Blood*. 2007;109:1316-1321.
53. Alchalby H, Badbaran A, Bacher U, et al. Screening and monitoring of MPL W515L mutation with real-time PCR in patients with myelofibrosis undergoing allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2010 Jan 11 [Epub ahead of print].
54. Keilholz U, Menssen HD, Gaiger A, et al. Wilms' tumour gene 1 (WT1) in human neoplasia. *Leukemia*. 2005;19:1318-1323.
55. Yang L, Han Y, Suarez SF, et al. A tumor suppressor and oncogene: the WT1 story. *Leukemia*. 2007;21:868-876.
56. Trka J, Kalinova M, Hrusak O, et al. Real-time quantitative PCR detection of WT1 gene expression in children with AML: prognostic significance, correlation with disease status and residual disease detection by flow cytometry. *Leukemia*. 2002;16:1381-1389.
57. Cilloni D, Gottardi E, De Micheli D, et al. Quantitative assessment of WT1 expression by real time quantitative PCR may be a useful tool for monitoring minimal residual disease in acute leukemia patients. *Leukemia*. 2002;16:2115-2121.
58. Willasch AM, Gruhn B, Coliva T, et al. Standardization of WT1 mRNA quantitation for minimal residual disease monitoring in childhood AML and implications of WT1 gene mutations: a European multicenter study. *Leukemia*. 2009;23: 1472-1479.
59. Inoue K, Ogawa H, Sonoda Y, et al. Aberrant overexpression of the Wilms tumor gene (WT1) in human leukemia. *Blood*. 1997; 89:1405-1412.
60. Menssen HD, Renkl HJ, Rodeck U, et al. Presence of Wilms' tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. *Leukemia*. 1995;9: 1060-1067.
61. Rosenfeld C, Cheever MA, Gaiger A. WT1 in acute leukemia, chronic myelogenous leukemia and myelodysplastic syndrome: therapeutic potential of WT1 targeted therapies. *Leukemia*. 2003;17:1301-1312.
62. Elisseeva OA, Oka Y, Tsuboi A, et al. Humoral immune responses against Wilms' tumor gene (WT1) product in patients with hematopoietic malignancies. *Blood*. 2002;99:3272-3279.
63. Algar E. A review of the Wilms' tumor 1 gene (WT1) and its role in hematopoiesis and leukemia. *J Hematother Stem Cell Res*. 2002;11:589-599.
64. Menssen HD, Siehl JM, Theil E. Wilms' tumor gene (wt1) expression as a panleukemia marker. *Int J Hematol*. 2002;76:103-109.
65. Drakos E, Rassidakis GZ, Tsioli P, Lai, et al. Differential expression of WT1 gene product in non-Hodgkin lymphomas. *Appl Immunohistochem Mol Morphol*. 2005;13:132-137.
66. Tamaki H, Ogawa H, Ohyashiki K, et al. The Wilms' tumor gene WT1 is a good marker for diagnosis of disease progression of myelodysplastic syndromes. *Leukemia*. 1999;13:393-399.
67. Ostergaard M, Olesen LH, Hasle H, et al. WT1 gene expression: an excellent tool for monitoring minimal residual disease in 70% of acute myeloid leukaemia patients— results from a single-centre study. *Br J Haematol*. 2004;125:590-600.
68. Osborne D, Frost L, Tobal K, et al. Elevated levels of WT1 transcripts in bone marrow harvests are associated with a high relapse risk in patients autografted for acute myeloid leukaemia. *Bone Marrow Transplant*. 2005;36:67-70.
69. Bader P, Niemeyer C, Weber G, et al. WT1 gene expression: useful marker for minimal residual disease in childhood myelodysplastic syndromes and juvenile myelo-monocytic leukemia? *Eur J Haematol*. 2004;73:25-28.
70. Cilloni D, Gottardi E, Messa F, et al. Significant correlation between the degree of WT1 expression and the International Prognostic Scoring System Score in patients with myelodysplastic syndromes. *J Clin Oncol*. 2003;21:1988-1995.
71. Hamalainen MM, Kairisto V, Juvonen V, et al. Wilms tumour gene 1 overexpression in bone marrow as a marker for minimal residual disease in acute myeloid leukaemia. *Eur J Haematol*. 2008;80:201-207.
72. Offit K, Burns JP, Cunningham I, et al. Cytogenetic analysis of chimerism and leukemia relapse in chronic myelogenous leukemia patients after T cell-depleted bone marrow transplantation. *Blood*. 1990;75:1346-1355.
73. Sparkes R. Cytogenetic analysis in human bone marrow transplantation. *Cancer Genet Cytogenet*. 1981;4:345-352.
74. Schattenberg A, de Witte T, Salden M, et al. Mixed hematopoietic chimerism after allogeneic transplantation with lymphocyte-depleted bone marrow is not associated with a higher incidence of relapse. *Blood*. 1989;73:1367-1372.
75. Schaap N, Schattenberg A, Bar B, et al. Red blood cell phenotyping is a sensitive technique for monitoring chronic myeloid leukaemia patients after T-cell-depleted bone marrow transplantation and after donor leucocyte infusion. *Br J Haematol*. 2000;108:116-125.
76. Schumm M, Feuchtinger T, Pfeiffer M, et al. Flow cytometry with anti HLA-antibodies: a simple but highly sensitive method for monitoring chimerism and minimal residual disease after HLA-mismatched stem cell transplantation. *Bone Marrow Transplant*. 2007;39:767-773.
77. Casarino L, Carbone C, Capucci MA, Izzi T, Ferrara GB. Analysis of chimerism after bone marrow transplantation using specific oligonucleotide probes. *Bone Marrow Transplant*. 1992; 10:165-170.
78. Knowlton RG, Brown VA, Braman JC, et al. Use of highly polymorphic DNA probes for genotypic analysis following bone marrow transplantation. *Blood*. 1986;68:378-385.
79. Yam PY, Petz LD, Knowlton RG, et al. Use of DNA restriction fragment length polymorphisms to document marrow engraftment and mixed hematopoietic chimerism following bone marrow transplantation. *Transplantation*. 1987;43:399-407.
80. Brunet S, Casals T, Madoz P, et al. DNA polymorphisms as implant markers in allogeneic bone marrow transplantation. Preliminary evaluation. *Med Clin (Barc)*. 1989;93:765-771.
81. Kogler G, Wolf HH, Heyll A, et al. Detection of mixed chimerism and leukemic relapse after allogeneic bone marrow transplantation in subpopulations of leucocytes by fluorescent in situ hybridization in combination with the simultaneous immunophenotypic analysis of interphase cells. *Bone Marrow Transplant*. 1995;15:41-48.
82. Petz LD, Yam P, Wallace RB, et al. Mixed hematopoietic chimerism following bone marrow transplantation for hematologic malignancies. *Blood*. 1987;70:1331-1337.
83. Bielorai B, Trakhtenbrot L, Amariglio N, et al. Multilineage hematopoietic engraftment after allogeneic peripheral blood stem cell transplantation without conditioning in SCID patients. *Bone Marrow Transplant*. 2004;34:317-320.
84. Thiele J, Wickenhauser C, Kvasnicka HM, et al. Mixed chimerism of erythro- and megakaryopoiesis following allogeneic bone marrow transplantation. *Acta Haematol*. 2003;109: 176-183.

85. Thiele J, Wickenhauser C, Kvasnicka HM, et al. Dynamics of lineage-restricted mixed chimerism following sex-mismatched allogeneic bone marrow transplantation. *Histol Histopathol*. 2003;18:557-574.
86. Seong CM, Giralt S, Kantarjian H, et al. Early detection of relapse by hypermetaphase fluorescence in situ hybridization after allogeneic bone marrow transplantation for chronic myeloid leukemia. *J Clin Oncol*. 2000;18:1831-1836.
87. Mulli KB. The unusual origin of the polymerase chain reaction. *Sci Am*. 1990;262:56-61.
88. Lawler M, McCann SR, Conneally E, et al. Chimaerism following allogeneic bone marrow transplantation: detection of residual host cells using the polymerase chain reaction. *Br J Haematol*. 1989;73:205-210.
89. Lawler M, Humphries P, McCann S. Evaluation of mixed chimerism by in vitro amplification of dinucleotide repeat sequences using the polymerase chaim reaction. *Blood*. 1991; 77:2504-2514.
90. Suttorp M, Schmitz N, Dreger P, et al. Monitoring of chimerism after allogeneic bone marrow transplantation with unmanipulated marrow by use of DNA polymorphisms. *Leukemia*. 1993;7:679-687.
91. van Leeuwen JE, van Tol MJ, Bodzinga BG, et al. Detection of mixed chimaerism in flow-sorted cell subpopulations by PCR-amplified VNTR markers after allogeneic bone marow transplantation. *Br J Haematol*. 1991;79:218-225.
92. Bertheas MF, Lafage M, Blaise D, et al. Mixed chimerism after allogeneic bone marrow transplantation for leukemias. *Bone Marrow Transplant*. 1990;6(Suppl 1):61-63.
93. Scharf SJ, Smith AG, Hansen JA, et al. Quantitative determination of bone marrow transplant engraftment using fluorescent polymerase chain reaction primers for human identity markers. *Blood*. 1995;85:1954-1963.
94. Oberkircher AR, Strout MP, Herzig GP, et al. Description of an efficient and highly informative method for the evaluation of hematopoietic chimerism following allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 1995;16: 695-702.
95. Kreyenberg H, Holle W, Mohrle S, et al. Quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection: the Tuebingen experience. *Leukemia*. 2003;17:237-240.
96. Hancock JP, Goulden NJ, Oakhill A, et al. Quantitative analysis of chimerism after allogeneic bone marrow transplantation using immunomagnetic selection and fluorescent microsatellite PCR. *Leukemia*. 2003;17:247-251.
97. Acquaviva C, Duval M, Mirebeau D, et al. Quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection: the Paris-Robert Debre experience. *Leukemia*. 2003;17:241-246.
98. Chalandon Y, Vischer S, Helg C, et al. Quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection: the Geneva experience. *Leukemia*. 2003;17:228-231.
99. Lo YM, Tein MS, Lau TK, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am J Hum Genet*. 1998;62:768-775.
100. Thiede C, Kellermann T, Schwerdtfeger R, et al. Real-time PCR for the SRY-gene allows sensitive and quantiative chimerism analysis after allogeneic blood stem cell transplantation: clinical results in 43 patients. *Bone Marrow Transplant*. 2003; 31(Suppl. 1):23.
101. Fehse B, Chukhlovin A, Kühlcke K, et al. Real-time quantitative Y chromosome-specific PCR (QYCS-PCR) for monitoring hematopoietic chimerism after sex-mismatched allogeneic stem cell transplantation. *J Hematother Stem Cell Res*. 2001; 10:419-425.
102. Sachidanandam R, Weissman D, Schmidt SC, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature*. 2001;409:928-933.
103. Wang DG, Fan JB, Siao CJ, et al. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science*. 1998;280:1077-1082.
104. Hochberg EP, Miklos DB, Neuberg D, et al. A novel rapid single nucleotide polymorphism (SNP)-based method for assessment of hematopoietic chimerism after allogeneic stem cell transplantation. *Blood*. 2003;101:363-369.
105. Alizadeh M, Bernard M, Danic B, et al. Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood*. 2002;99:4618-4625.
106. Maas F, Schaap N, Kolen S, et al. Quantification of donor and recipient hemopoietic cells by real-time PCR of single nucleotide polymorphisms. *Leukemia*. 2003;17:621-629.
107. Fredriksson M, Barbany G, Liljedahl U, et al. Assessing hematopoietic chimerism after allogeneic stem cell transplantation by multiplexed SNP genotyping using microarrays and quantitative analysis of SNP alleles. *Leukemia*. 2004;18:255-266.
108. Thiede C, Florek M, Bornhauser M, et al. Rapid quantification of mixed chimerism using multiplex amplification of short tandem repeat markers and fluorescence detection. *Bone Marrow Transplant*. 1999;23:1055-1060.
109. de Weger RA, Tilanus MG, Scheidel KC, et al. Monitoring of residual disease and guided donor leucocyte infusion after allogeneic bone marrow transplantation by chimaerism analysis with short tandem repeats. *Br J Haematol*. 2000;110:647-653.
110. Lion T, Daxberger H, Dubovsky J, et al. Analysis of chimerism within specific leukocyte subsets for detection of residual or recurrent leukemia in pediatric patients after allogeneic stem cell transplantation. *Leukemia*. 2001;15:307-310.
111. Willasch A, Schneider G, Reincke BS, et al. Sequence polymorphism systems for quantitative real-time polymerase chain reaction to characterize hematopoietic chimerism-high informativity and sensitivity as well as excellent reproducibility and precision of measurement. *Lab Hematol*. 2007;13:73-84.
112. Roux E, Abdi K, Speiser D, et al. Characterization of mixed chimerism in patients with chronic myeloid leukemia transplanted with T-cell-depleted bone marrow: involvement of different hematologic lineages before and after relapse. *Blood*. 1993;81:243-248.
113. Fernández-Avilés F, Urbano-Ispizua A, Aymerich M, et al. Serial quantification of lymphoid and myeloid mixed chimerism using multiplex PCR amplification of short tandem repeat-markers predicts graft rejection and relapse, respectively, after allogeneic transplantation of CD34+ selected cells from peripheral blood. *Leukemia*. 2003;17:613-620.
114. Kröger N, Zagrivnaja M, Schwartz S, et al. Kinetics of plasmacell chimerism after allogeneic stem cell transplantation by highly-sensitive real-time PCR based on sequence polymorphism and its value to quantify minimal residual disease in patients with multiple myeloma. *Exp Hematol*. 2006;34:688-694.
115. Antin JH, Childs R, Filipovich AH, et al. Establishment of complete and mixed donor chimerism after allogeneic lymphohematopoietic transplantation. *Biol Blood Marrow Transplant*. 2001;7:473-485.
116. Edelstyn GA, Gillespie PJ, Grebbell FS. The radiological demonstration of osseous metastases. Experimental observations. *Clin Radiol*. 1967;18:158-162.
117. Armitage JO. Staging non-Hodgkin's lymphoma. *CA Cancer J Clin*. 2005;55:368-376.
118. Lister TA, Crowther D, Sutcliffe SB, et al. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Coswolds meeting. *J Clin Oncol*. 1989;7:1630-1636.
119. Jhanwar YS, Straus DJ. The role of PET in lymphoma. *J Nucl Med*. 2006;47:1326-1334.

120. Vinnicombe SJ, Reznek RH. Computerised tomography in the staging of Hodgkin's disease and non-Hodgkin's lymphoma. *Eur J Nucl Med Mol Imaging*. 2003;30:S42-S55.
121. Mahnken AH, Wildberger JE, Gehbauer G, et al. Multidetector CT of the spine in multiple myeloma: comparison with MR imaging and radiography. *AJR*. 2002;180:1429-1436.
122. Libshitz HI, Malthouse SR, Cunningham D, et al. Multiple myeloma: appearnace at MR imaging. *Radiology*. 1992;182: 833-837.
123. Rohren EM, Turkington TG, Coleman RE. Clinical applications of PET in oncology. *Radiology*. 2004;231:305-332.
124. Von Schulthess GK, Steinert HC, Hany TF. Integrated PET/CT: current applications and future directions. *Radiology*. 2006; 238:405-422.
125. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. *J Clin Oncol*. 2007;25:579-586.
126. Bruzzi JF, Macapinlac H, Apostolia M, et al. Detection of Richter's transformation of chronic lymphocytic leukemia by PET/CT. *J Nucl Med*. 2006;47:1267-1273.
127. Boellaard R, O'Doherty MJ, Weber WA, et al. FDG PET and PET/CT: EANM procedure guidelines for tumour PET imaging: version 1.0. *Eur J Nucl Med Mol Imaging*. 2010;37: 181-200.
128. Wahl RL, Jacene H, Kasamon Y, et al. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. *J Nucl Med*. 2009;50(Suppl):122S-150S.
129. Hutchings M, Barrington SF. PET/CT for therapy response assessment in lymphoma. *J Nucl Med*. 2009;50(Suppl):21S-30S.
130. Hermann K, Weider HA, Buck AK, et al. Early response assessment using 3′-deoxy-3′-[18F] fluorothymidine-positron emission tomography in high-grade non-Hodgkin's lymphoma. *Clin Cancer Res*. 2007;13:3552-3558.
131. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *J Clin Oncol*. 2003;21:4642-4649.
132. Marcucci G, Mrozek K, Ruppert AS, et al. Abnormal cytogenetics at date of morphologic complete remission predicts short overall and disease-free survival, and higher relapse rate in adult acute myeloid leukemia: results from cancer and leukemia group B study 8461. *J Clin Oncol*. 2004;22:2410-2418.
133. El-Rifai W, Ruutu T, Elonen E, et al. Prognostic value of metaphase-fluorescence in situ hybridization in follow-up of patients with acute myeloid leukemia in remission. *Blood*. 1997;89:3330-3334.
134. Bader P, Kreyenberg H, Hoelle W, et al. Increasing mixed chimerism defines a high-risk group of childhood acute myelogenous leukemia patients after allogeneic stem cell transplantation where pre-emptive immunotherapy may be effective. *Bone Marrow Transplant*. 2004;33:815-821.
135. Huisman C, de Weger RA, de Vries L, et al. Chimerism analysis within 6 months of allogeneic stem cell transplantation predicts relapse in acute myeloid leukemia. *Bone Marrow Transplant*. 2007;39:285-291.
136. Zeiser R, Spyridonidis A, Wasch R, et al. Evaluation of immunomodulatory treatment based on conventional and lineage-specific chimerism analysis in patients with myeloid malignancies after myeloablative allogeneic hematopoietic cell transplantation. *Leukemia*. 2005;19:814-821.
137. Leroy H, de Botton S, Grardel-Duflos N, et al. Prognostic value of real-time quantitative PCR (RQ-PCR) in AML with t(8;21). *Leukemia*. 2005;19:367-372.
138. Elmaagacli AH, Beelen DW, Kroll M, et al. Detection of CBFbeta/MYH11 fusion transcripts in patients with inv(16) acute myeloid leukemia after allogeneic bone marrow or peripheral blood progenitor cell transplantation. *Bone Marrow Transplant*. 1998;21:159-166.
139. Gorello P, Cazzaniga G, Alberti F, et al. Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. *Leukemia*. 2006;20:1103-1108.
140. Chou WC, Tang JL, Wu SJ, et al. Clinical implications of minimal residual disease monitoring by quantitative polymerase chain reaction in acute myeloid leukemia patients bearing nucleophosmin (NPM1) mutations. *Leukemia*. 2007;21: 998-1004.
141. Barragan E, Pajuelo JC, Ballester S, et al. Minimal residual disease detection in acute myeloid leukemia by mutant nucleophosmin (NPM1): comparison with WT1 gene expression. *Clin Chim Acta*. 2008;395:120-123.
142. Papadaki C, Dufour A, Seibl M, et al. Monitoring minimal residual disease in acute myeloid leukaemia with NPM1 mutations by quantitative PCR: clonal evolution is a limiting factor1. *Br J Haematol*. 2009;144:517-523.
143. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*. 2005;352:254-266.
144. Dohner K, Schlenk RF, Habdank M, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood*. 2005;106:3740-3746.
145. Schnittger S, Schoch C, Dugas M, et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood*. 2002;100:59-66.
146. Scholl S, Loncarevic IF, Krause C, et al. Minimal residual disease based on patient specific Flt3-ITD and -ITT mutations in acute myeloid leukemia. *Leuk Res*. 2005;29:849-853.
147. Cloos J, Goemans BF, Hess CJ, et al. Stability and prognostic influence of FLT3 mutations in paired initial and relapsed AML samples. *Leukemia*. 2006;20:1217-1220.
148. Schnittger S, Schoch C, Kern W, et al. FLT3 length mutations as marker for follow-up studies in acute myeloid leukaemia. *Acta Haematol*. 2004;112:68-78.
149. Elmaagacli AH. Molecular methods used for detection of minimal residual disease following hematopoietic stem cell transplantation in myeloid disorders. *Methods Mol Med*. 2007;134: 161-178.
150. Shimoni A, Nagler A. Clinical implications of minimal residual disease monitoring for stem cell transplantation after reduced intensity and nonmyeloablative conditioning. *Acta Haematol*. 2004;112:93-104.
151. Falini B, Nicoletti I, Martelli MF, et al. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. *Blood*. 2007;109:874-885.
152. Abu-Duhier FM, Goodeve AC, Wilson GA, et al. FLT3 internal tandem duplication mutations in adult acute myeloid leukaemia define a high-risk group. *Br J Haematol*. 2000;111: 190-195.
153. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood*. 2002;99:4326-4335.
154. Yamamoto Y, Kiyoi H, Nakano Y, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood*. 2001;97:2434-2439.
155. Bacher U, Haferlach C, Kern W, et al. Prognostic relevance of FLT3-TKD mutations in AML: the combination matters—an analysis of 3082 patients. *Blood*. 2008;111:2527-2537.
156. Mead AJ, Linch DC, Hills RK, et al. FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. *Blood*. 2007;110:1262-1270.
157. Scholl S, Loncarevic IF, Krause C, et al. Analyses of minimal residual disease based on Flt3 mutations in allogeneic peripheral blood stem cell transplantation. *J Cancer Res Clin Oncol*. 2005;131:279-283.

158. Schlenk RF, Dohner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med*. 2008;358:1909-1918.
159. Schichman SA, Caligiuri MA, Strout MP, et al. ALL-1 tandem duplication in acute myeloid leukemia with a normal karyotype involves homologous recombination between Alu elements. *Cancer Res*. 1994;54:4277-4280.
160. Schnittger S, Kinkelin U, Schoch C, et al. Screening for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. *Leukemia*. 2000;14:796-804.
161. Dohner K, Tobis K, Ulrich R, et al. Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: a study of the Acute Myeloid Leukemia Study Group Ulm. *J Clin Oncol*. 2002;20:3254-3261.
162. Schoch C, Schnittger S, Klaus M, et al. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *Blood*. 2003;102:2395-2402.
163. Weisser M, Kern W, Schoch C, et al. Risk assessment by monitoring expression levels of partial tandem duplications in the MLL gene in acute myeloid leukemia during therapy. *Haematologica*. 2005;90:881-889.
164. Ogawa H, Tamaki H, Ikegame K, et al. The usefulness of monitoring WT1 gene transcripts for the prediction and management of relapse following allogeneic stem cell transplantation in acute type leukemia. *Blood*. 2003;101:1698-1704.
165. Candoni A, Tiribelli M, Toffoletti E, et al. Quantitative assessment of WT1 gene expression after allogeneic stem cell transplantation is a useful tool for monitoring minimal residual disease in acute myeloid leukemia. *Eur J Haematol*. 2009;82:61-68.
166. Jacobsohn DA, Tse WT, Chaleff S, et al. High WT1 gene expression before haematopoietic stem cell transplant in children with acute myeloid leukaemia predicts poor event-free survival. *Br J Haematol*. 2009;146:669-674.
167. Langer C, Radmacher MD, Ruppert AS, et al. High BAALC expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: a Cancer and Leukemia Group B (CALGB) study. *Blood*. 2008;111:5371-5379.
168. Campana D. Determination of minimal residual disease in leukaemia patients. *Br J Haematol*. 2003;121:823-838.
169. Kern W, Voskova D, Schoch C, et al. Determination of relapse risk based on assessment of minimal residual disease during complete remission by multiparameter flow cytometry in unselected patients with acute myeloid leukemia. *Blood*. 2004;104:3078-3085.
170. Griesinger F, Piro-Noack M, Kaib N, et al. Leukaemia-associated immunophenotypes (LAIP) are observed in 90% of adult and childhood acute lymphoblastic leukaemia: detection in remission marrow predicts outcome. *Br J Haematol*. 1999;105:241-255.
171. Coustan-Smith E, Ribeiro RC, Rubnitz JE, et al. Clinical significance of residual disease during treatment in childhood acute myeloid leukaemia. *Br J Haematol*. 2003;123:243-322.
172. Nagler A, Condiotti R, Rabinowitz R, et al. Detection of minimal residual disease (MRD) after bone marrow transplantation (BMT) by multi-parameter flow cytometry (MPFC). *Med Oncol*. 1999;16:177-187.
173. Wells DA, Sale GE, Shulman HM, et al. Multidimensional flow cytometry of marrow can differentiate leukemic from normal lymphoblasts and myeloblasts after chemotherapy and bone marrow transplantation. *Am J Clin Pathol*. 1998;110:84-94.
174. Ito S, Ishida Y, Murai K, et al. Flow cytometric analysis of aberrant antigen expression of blasts using CD45 blast gating for minimal residual disease in acute leukemia and high-risk myelodysplastic syndrome. *Leuk Res*. 2001;25:205-211.
175. Perez-Simon JA, Caballero D, Diez-Campelo M, et al. Chimerism and minimal residual disease monitoring after reduced intensity conditioning (RIC) allogeneic transplantation. *Leukemia*. 2002;16:1423-1431.
176. Diez-Campelo M, Perez-Simon JA, Perez J, et al. Minimal residual disease monitoring after allogeneic transplantation may help to individualize post-transplant therapeutic strategies in acute myeloid malignancies. *Am J Hematol*. 2009;84:149-152.
177. Voskova D, Schoch C, Schnittger S, et al. Stability of leukemia-associated aberrant immunophenotypes in patients with acute myeloid leukemia between diagnosis and relapse: comparison with cytomorphologic, cytogenetic, and molecular genetic findings. *Cytometry B Clin Cytom*. 2004;62:25-38.
178. Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. *Blood*. 2006;108:419-425.
179. Patmasiriwat P, Fraizer G, Kantarjian H, et al. WT1 and GATA1 expression in myelodysplastic syndrome and acute leukemia. *Leukemia*. 1999;13:891-900.
180. Tamura K, Kanazawa T, Suzuki M. Successful rapid discontinuation of immunosuppressive therapy at molecular relapse after allogeneic bone marrow transplantation in a pediatric patient with myelodysplastic syndrome. *Am J Hematol*. 2006;81:139-141.
181. Chen CY, Lin LI, Tang JL, et al. RUNX1 gene mutation in primary myelodysplastic syndrome—the mutation can be detected early at diagnosis or acquired during disease progression and is associated with poor outcome. *Br J Haematol*. 2007;139:405-414.
182. Christiansen DH, Andersen MK, Pedersen-Bjergaard J. Mutations of AML1 are common in therapy-related myelodysplasia following therapy with alkylating agents and are significantly associated with deletion or loss of chromosome arm 7q and with subsequent leukemic transformation. *Blood*. 2004;104:1474-1481.
183. Yanada M, Matsuo K, Suzuki T, et al. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations for acute myeloid leukemia: a meta-analysis. *Leukemia*. 2005;19:1345-1349.
184. Shih LY, Huang CF, Wang PN, et al. Acquisition of FLT3 or N-ras mutations is frequently associated with progression of myelodysplastic syndrome to acute myeloid leukemia. *Leukemia*. 2004;18:466-475.
185. Bacher U, Haferlach T, Kern W, et al. A comparative study of molecular mutations in 381 patients with myelodysplastic syndrome and in 4130 patients with acute myeloid leukemia. *Haematologica*. 2007;92:744-752.
186. Zhang Y, Zhang M, Yang L, et al. NPM1 mutations in myelodysplastic syndromes and acute myeloid leukemia with normal karyotype. *Leuk Res*. 2007;31:109-111.
187. Stetler-Stevenson M, Arthur DC, Jabbour N, et al. Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. *Blood*. 2001;98:979-987.
188. Della Porta MG, Malcovati L, Invernizzi R, et al. Flow cytometry evaluation of erythroid dysplasia in patients with myelodysplastic syndrome. *Leukemia*. 2006;20:549-555.
189. Van de Loosdrecht A, Alhan C, Bene MC, et al. Standardization of flow cytometry in myelodysplastic syndromes: report from the first ELNet working conference on flow cytometry in MDS. *Haematologica*. 2009;94:1124-1134.
190. Wells DA, Benesch M, Loken MR, et al. Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. *Blood*. 2003;102:394-403.
191. Brisco MJ, Condon J, Hughes E, et al. Outcome prediction in childhood acute lymphoblastic leukaemia by molecular quantification of residual disease at the end of induction. *Lancet*. 1994;343:196-200.

192. Cavé H, van der Werff ten Bosch J, Suciu S, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer—Childhood Leukemia Cooperative Group. *N Engl J Med*. 1998;339:591-598.
193. Coustan-Smith E, Behm FG, Sanchez J, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. *Lancet*. 1998;351:550-554.
194. Szczepanski T, Orfao A, van der Velden VH, et al. Minimal residual disease in leukaemia patients. *Lancet Oncol*. 2001;2:409-417.
195. an Dongen JJ, Seriu T, Panzer-Grümayer ER, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. *Lancet*. 1998;352. 731–1738.
196. Pui CH, Campana D. New definition of remission in childhood acute lymphoblastic leukemia. *Leukemia*. 2000;14:783-785.
197. Schrappe M, Beier R, Burger B. New treatment strategies in childhood acute lymphoblastic leukaemia. *Best Pract Res Clin Haematol*. 2002;15:729-740.
198. Gameiro P, Mortuza FY, Hoffbrand, et al. Minimal residual disease monitoring in adult T-cell acute lymphoblastic leukemia: a molecular based approach using T-cell receptor G and D gene rearrangements. *Haematologica*. 2002;87:1126-1134.
199. Specchia G, Liso A, Pannunzio A, et al. Molecular detection of minimal residual disease is associated with early relapse in adult acute lymphoblastic leukemia. *Haematologica*. 2004;89: 1271-1273.
200. Toubai T, Tanaka J, Ota S, et al. Minimal residual disease (MRD) monitoring using rearrangement of T-cell receptor and immunoglobulin H gene in the treatment of adult acute lymphoblastic leukemia patients. *Am J Hematol*. 2005;80: 181-187.
201. Mortuza FY, Papaioannou M, Moreira IM, et al. Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. *J Clin Oncol*. 2002;20:1094-1104.
202. Brüggemann M, Raff T, Flohr T, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. *Blood*. 2006;107: 1116-1123.
203. Krampera M, Vitale A, Vincenzi C, et al. Outcome prediction by immunophenotypic minimal residual disease detection in adult T-cell acute lymphoblastic leukaemia. *Br J Haematol*. 2003;120:74-79.
204. Holowiecki J, Krawczyk-Kulis M, Giebel S, et al. Status of minimal residual disease after induction predicts outcome in both standard and high-risk Ph-negative adult acute lymphoblastic leukaemia. The Polish Adult Leukemia Group ALL 4-2002 MRD Study. *Br J Haematol*. 2008;142:227-237.
205. Raff T, Gökbuget N, Lüschen S, et al. Molecular relapse in adult standard-risk ALL patients detected by prospective MRD monitoring during and after maintenance treatment: data from the GMALL 06/99 and 07/03 trials. *Blood*. 2007; 109:910-915.
206. Bassan R, Spinelli O, Oldani E, et al. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). *Blood*. 2009;113:4153-4162.
207. Bruggemann M, Droese J, Raff T. the prognostic significance of minimal residual disease in adult standard risk patients with acute lymphoblastic leukemia. *Blood*. 2001;98:14a.
208. Coustan-Smith E, Sancho J, Hancock ML, et al. Use of peripheral blood instead of bone marrow to monitor residual disease in children with acute lymphoblastic leukemia. *Blood*. 2002;100: 2399-2402.
209. van der Velden VH, Jacobs DC, Wijkhuijs AJ, et al. Minimal residual disease levels in bone marrow and peripheral blood are comparable in children with T cell acute lymphoblastic leukemia (ALL), but not in precursor-B-ALL. *Leukemia*. 2002;16: 1432-1436.
210. Imashuku S, Terui K, Matsuyama T, et al. Multi-institutional collaborative study in Japan. Lack of clinical utility of minimal residual disease detection in allogeneic stem cell recipients with childhood acute lymphoblastic leukemia: multi-institutional collaborative study in Japan. *Bone Marrow Transpant*. 2003; 31:1127-1135.
211. Bader P, Hancock J, Kreyenberg H, et al. Minimal residual disease (MRD) status prior to allogeneic stem cell transplantation is a powerful predictor for post-transplant outcome in children with ALL. *Leukemia*. 2002;16:1668-1672.
212. Campana D. Minimal residual disease in acute lymphoblastic leukemia. *Semin Hematol*. 2009;46:100-106.
213. Knechtli CJ, Goulden NJ, Hancock JP, et al. Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. *Blood*. 1998;92:4072-4079.
214. Bader P, Kreyenberg H, Henze GH, Eckert C, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. *J Clin Oncol*. 2009;27:377-384.
215. Spinelli O, Peruta B, Tosi M, et al. Clearance of minimal residual disease after allogeneic stem cell transplantation and the prediction of the clinical outcome of adult patients with high-risk acute lymphoblastic leukemia. *Haematologica*. 2007; 92:612-618.
216. Gehly GB, Bryant EM, Lee AM, et al. Chimeric BCR-abl messenger RNA as a marker for minimal residual disease in patients transplanted for Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood*. 1991;78:458-465.
217. Miyamura K, Tanimoto M, Morishima Y, et al. Detection of Philadelphia chromosome-positive acute lymphoblastic leukemia by polymerase chain reaction: possible eradication of minimal residual disease by marrow transplantation. *Blood*. 1992; 79:1366-1370.
218. Radich J, Gehly G, Lee A, et al. Detection of bcr-abl transcripts in Philadelphia chromosome-positive acute lymphoblastic leukemia after marrow transplantation. *Blood*. 1997;89: 2602-2609.
219. Radich J, Ladne P, Gooley T. Polymerase chain reaction-based detection of minimal residual disease in acute lymphoblastic leukemia predicts relapse after allogeneic BMT. *Biol Blood Marrow Transplant*. 1995;1:24-31.
220. Dombret H, Gabert J, Boiron JM, et al. Outcome of treatment in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia—results of the prospective multicenter LALA-94 trial. *Blood*. 2002;100:2357-2366.
221. Lee S, Kim YJ, Min CK, et al. The effect of first-line imatinib interim therapy on the outcome of allogeneic stem cell transplantation in adults with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood*. 2005;105:3449-3457.
222. Yanada M, Takeuchi J, Sugiura I, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. *J Clin Oncol*. 2006;24:460-466.
223. Ottmann OG, Wassmann B, Pfeifer H, et al. Imatinib compared with chemotherapy as front-line treatment of elderly patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL). *Cancer*. 2007;109:2068-2076.
224. Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. *Blood*. 2004;103:4396-4407.
225. Towatari M, Yanada M, Usui N, et al. Combination of intensive chemotherapy and imatinib can rapidly induce high-quality complete remission for a majority of patients with newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia. *Blood*. 2004;104:3507-3512.
226. Lee S, Kim DW, Kim YJ, et al. Minimal residual disease-based role of imatinib as a first-line interim therapy prior to allogeneic

stem cell transplantation in Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood*. 2003;102:3068-3070.
227. Lee S, Kim YJ, Chung NG, et al. The extent of minimal residual disease reduction after the first 4-week imatinib therapy determines outcome of allogeneic stem cell transplantation in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Cancer*. 2009;115:561-570.
228. Bader P, Hölle W, Klingebiel T, et al. Mixed hematopoietic chimerism after allogeneic bone marrow transplantation: the impact of quantitative PCR analysis for prediction of relapse and graft rejection in children. *Bone Marrow Transplant*. 1997; 19:697-702.
229. Formánková R, Sedlácek P, Krsková L, et al. Chimerism-directed adoptive immunotherapy in prevention and treatment of post-transplant relapse of leukemia in childhood. *Haematologica*. 2003;88:117-118.
230. Gorczyñska E, Turkiewicz D, Toporski J, et al. Prompt initiation of immunotherapy in children with an increasing number of autologous cells after allogeneic HCT can induce complete donor-type chimerism: a report of 14 children. *Bone Marrow Transplant*. 2004;33:211-217.
231. Ramírez M, Díaz MA, García-Sánchez F, et al. Chimerism after allogeneic hematopoietic cell transplantation in childhood acute lymphoblastic leukemia. *Bone Marrow Transplant*. 1996; 18:1161-1165.
232. Guimond M, Busque L, Baron C, et al. Relapse after bone marrow transplantation: evidence for distinct immunological mechanisms between adult and paediatric populations. *Br J Haematol*. 2000;109:130-137.
233. Barrios M, Jiménez-Velasco A, Román-Gómez J, et al. Chimerism status is a useful predictor of relapse after allogeneic stem cell transplantation for acute leukemia. *Haematologica*. 2003;88: 801-810.
234. Bader P, Stoll K, Huber A, et al. Characterization of lineages specific chimerism in patients with acute leukaemia and myelodysplastic syndrome after allogeneic stem cell transplantation before and after relapse. *Br J Haematol*. 2000;108:761-768.
235. Bader P, Kreyenberg H, Hoelle W, et al. Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukemia after allogeneic stem-cell transplantation: possible role for preemptive immunotherapy? *J Clin Oncol*. 2004;22:1696-1705.
236. Pulsipher MA, Bader P, Klingebiel T, et al. Allogeneic transplantation for pediatric acute lymphoblastic leukemia: the emerging role of peritransplantation minimal residual disease/chimerism monitoring and novel chemotherapeutic, molecular, and immune approaches aimed at preventing relapse. *Biol Blood Marrow Transplant*. 2008;15:62-71.
237. Knechtli CJ, Goulden NJ, Hancock JP, et al. Minimal residual disease status as a predictor of relapse after allogeneic bone marrow transplantation for children with acute lymphoblastic leukaemia. *Br J Haematol*. 1998;102:860-871.
238. Sánchez J, Serrano J, Gómez P, et al. Clinical value of immunological monitoring of minimal residual disease in acute lymphoblastic leukaemia after allogeneic transplantation. *Br J Haematol*. 2002;116:686-694.
239. Uzunel M, Jaksch M, Mattsson J, et al. Minimal residual disease detection after allogeneic stem cell transplantation is correlated to relapse in patients with acute lymphoblastic leukaemia. *Br J Haematol*. 2003;122:788-794.
240. Bader P. Predictive value of MRD monitoring after allogeneic SCT in relapsed childhood acute lymphoblastic leukaemia: analysis of the ALL-REZ BFM Group. *Bone Marrow Transplant*. 2009;43:40.
241. Thiede C, Lutterbeck K, Oelschlägel U, et al. Detection of relapse by sequential monitoring of chimerism in circulating CD34+ cells. *Ann Hematol*. 2002;81(Suppl 2):S27-S28.
242. Winiarski J, Mattsson J, Gustafsson A, et al. Engraftment and chimerism, particularly of T- and B-cells, in children undergoing allogeneic bone marrow transplantation. *Pediatr Transplant*. 1998;2:150-156.
243. Sierra J, Radich J, Hansen JA, et al. Marrow transplants from unrelated donors for treatment of Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood*. 1997;90:1410-1414.
244. Wassmann B, Pfeifer H, Stadler M, et al. Early molecular response to posttransplantation imatinib determines outcome in MRD+ Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood*. 2005;106:458-463.
245. van Leeuwen JE, van Tol MJ, Joosten AM, et al. Mixed T-lymphoid chimerism after allogeneic bone marrow transplantation for hematologic malignancies of children is not correlated with relapse. *Blood*. 1993;82:1921-1928.
246. Roy DC, Tantravahi R, Murray C, et al. Natural history of mixed chimerism after bone marrow transplantation with CD6-depleted allogeneic marrow: a stable equilibrium. *Blood*. 1990;75:296-304.
247. Bader P, Frey A, Beck j, et al. Serial and quantitative analysis of mixed hematopoietic chimerism by PCR in patients with acute leukemias allows the prediction of relapse after allogeneic BMT. *Bone Marrow Transplant*. 1998;21:487-495.
248. Horn B, Soni S, Khan S, et al. Feasibility study of preemptive withdrawal of immunosuppression based on chimerism testing in children undergoing myeloablative allogeneic transplantation for hematologic malignancies. *Bone Marrow Transplant*. 2009;43:469-476.
249. Uzunel M, Mattsson J, Jaksch M, et al. The significance of graft-versus-host disease and pretransplantation minimal residual disease status to outcome after allogeneic stem cell transplantation in patients with acute lymphoblastic leukemia. *Blood*. 2001;98:1982-1984.
250. Sramkova L, Muzikova K, Fronkova E, et al. Detectable minimal residual disease before allogeneic hematopoietic stem cell transplantation predicts extremely poor prognosis in children with acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2007;48:93-100.